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Epicardial fat: the role of testosterone and lipid metabolism in a cohort of patients with Klinefelter syndrome

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ABSTRACT

Context: Klinefelter syndrome (KS), in which subjects have additional copies of X chromosomes, is the most common male sex chromosome abnormality, with a prevalence of 1 in 660 and an incidence of about 1 in 500–700 newborns. Its sign and symptoms include infertility, generally low testosterone levels, and an increased prevalence of obesity and metabolic syndrome. Epicardial fat thickness (EFT) reflects visceral adiposity rather than general obesity.
Objective: The aim of this study was to analyze echocardiographic EFT in a cohort of patients with KS in comparison with non-obese and obese euploid controls, and to evaluate its correlation with biochemical parameters.
Design, setting and participants: Two hundred and twenty-one KS patients referred to our Rare Endocrine Diseases clinic and 77 age-matched controls underwent Doppler echocardiography and a full investigation of anthropometric and body composition, Serum levels of total testosterone (T), estradiol (E2), sex hormone binding globulin (SHBG), fasting plasma glucose, insulin, cholesterol and triglycerides were obtained. All participants underwent dual energy X-ray absorptiometry (DEXA) scan to assess truncal body fat (TrBF).
Main outcome measure: EFT, body composition and metabolic parameters in KS patients and how they are affected by genotype.
Results: EFT was greater in KS patients than in healthy non-obese (NOb) controls, but lower than in obese (OB) controls. When KS patients were divided into groups (hypogonadal; eugonadal; receiving testosterone replacement therapy [TRT]), EFT was greater in hypogonadal patients than in NOb controls and eugonadal patients, but showed no difference from the OB controls or TRT patients. Hypogonadal patients showed increased TrBF in comparison with NOb controls and eugonadal and TRT patients, and similar TrBF to OB controls. As expected, there was a strong correlation between BMI and EFT in both KS patients and controls ($P < 0.0001$). In contrast, there was a strong inverse correlation between testosterone and EFT in the control group, but not in KS patients. EFT was significantly correlated with TrBF in both populations ($P < 0.0001$). Multivariate analyses showed that the major determinants of both EFT and TrBF were BMI and the presence of KS itself. Testosterone and triglycerides were not included as variables in the models.
Conclusion: EFT in hypogonadal KS subjects was similar to that of the obese eugonadal controls. Even though there was a direct correlation between BMI and EFT in both populations, the influence of TrBF on EFT was stronger. The presence of the supernumerary X chromosome appeared to be one of the strongest determinants of EFT and TrBF, independent of testosterone levels.

1. Introduction

Klinefelter syndrome (KS) is the most common sex chromosome disorder in males, with an estimated prevalence of 1 case per 660 newborns (Table 1) [1–3]. Its clinical features commonly include hypergonadotropic hypogonadism, small testes, and azoospermia [4], but it may also be associated with obesity, type 2 diabetes mellitus, dyslipidemia, and an increased risk of cardiovascular disease [5–8]. It has been shown that metabolic syndrome has a higher prevalence in patients with KS than in the general population (42% in KS vs 10% in controls) [9–11]. Adiposity, above all truncal body fat (TrBF), was found to be the strongest predictor of metabolic syndrome and insulin sensitivity in KS patients [12–14]. The

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Table 1
Definition of the abbreviations used in the manuscript.

Acronym	Meaning
KS	Klinefelter syndrome
EFT	Epicardial fat thickness
T	Testosterone
E2	Estradiol
SHBG	Sex hormone binding globulin
DEXA	Dual energy X-ray absorptiometry
TrBF	Truncal body fat
TRT	Testosterone replacement therapy
NOb	Non-obese euploid subjects
OB	Obese euploid subjects

impact of hypogonadism on the presence of metabolic syndrome or insulin sensitivity disappeared when the data were controlled for TrBF, thus indicating that there is no direct unique dependent correlation between testosterone (T) and metabolic syndrome. A vicious circle may explain the association between T levels and metabolic disorder in KS, with hypogonadism influencing body composition by causing an increase in body (especially intra-abdominal) fat and subsequent modification of carbohydrates and fats, in turn causing insulin resistance, which further aggravates the hypogonadism [14,15].

Epicardial adipose tissue has recently emerged as a new risk factor and active player in metabolic and cardiovascular diseases. Epicardial adipose tissue is visceral fat bordering the heart, and lies between the myocardium and the visceral pericardial tissue [16–18]. Given its anatomic proximity to the heart, it may interact locally and modulate the coronary arteries by secretion of proinflammatory adipokines. It has been demonstrated that increased epicardial fat thickness (EFT) is independently correlated with adverse cardiovascular events. It has also been suggested that EFT may play an independent role in the development and progression of obesity and metabolic disorders that are directly linked to cardiac abnormalities [17]. It was recently reported that EFT is higher in subjects with early atherosclerosis than in subjects without coronary calcium, but similar to EFT in subjects with advanced atherosclerosis, suggesting that the role of epicardial fat as a predictive factor for cardiovascular disease is not restricted to subjects with advanced vascular impairment [19].

There is evidence that EFT measurement may complement the prognostic information offered by the coronary artery calcification score without the need for extra radiation exposure or administration of contrast media [20,21]. In addition, there are numerous studies in the literature about how EFT responds to treatment, with both weight loss and bariatric surgery reported to be significantly correlated with a reduction in EFT [22]. It has also been reported that oral antidiabetic medications are strongly associated with reduced EFT, opening new prospects for the clinical use of GLP-1 analogs [23]. In our Department, Francomano et al described a similar significant reduction with testosterone replacement therapy (TRT) in a cohort of hypogonadal obese patients [24]. They demonstrated that TRT in their population was safe and improved both cardiometabolic and hormonal parameters and body composition. Given its particular properties and rapid responsiveness, epicardial adipose tissue is a potential new therapeutic target for treatments modulating adipose tissue.

Left ventricular diastolic dysfunction and other cardiac anomalies have been described as very common in KS and not reversed by TRT [25,26]. However, it has been shown that TRT affects body composition, inducing an increase in lean mass and a reduction in fat mass [15].

Given the total lack of published data on TRT in KS subjects, the main aim of this study was to evaluate epicardial adipose tissue in KS patients, in order to detect any difference in comparison with age-matched euploid men. The second aim was to confirm the expected relationship between metabolic blood parameters, TrBF and EFT in KS patients and to understand how they are affected by testosterone.

2. Materials and methods

2.1. Subjects

We enrolled 221 men with KS aged 34.2 ± 12 years attending our Center for Rare Diseases (Section of Medical Pathophysiology and Endocrinology), Department of Experimental Medicine (Sapienza University of Rome) and 77 euploid men (aged 34 ± 11 years) attending our outpatient clinic in the same department between January 2013 and December 2017. The inclusion criteria were age above 18 years and no clinical history of cardiovascular disease. All KS patients had the classic 47, XXY karyotype, as verified by chromosome analysis of peripheral blood lymphocytes. Karyotypes were established on 40 metaphases from each patient.

The participants were examined in the morning after an overnight fast. After blood collection, serum and plasma were immediately separated and stored at −80 °C in multiple vials for later analysis. Subject evaluation included a complete medical history (pubertal history, lifestyle, physical activity, smoking, alcohol use).

All KS patients and control subjects received verbal and written information on the study before giving their written informed consent. The protocol was approved by the University's Institutional Ethics Committee.

Given the broad range of body weight in the euploid controls, they were divided into two subgroups by BMI: NOb (not obese), with a BMI <30 kg/m^2, and OB (obese), with a BMI ≥30 kg/m^2, as indicated in Table 2. The KS cohort was divided into three subgroups (Table 3) on the basis of T levels and TRT, as follows: eugonadal [KS patients with normal T levels (≥12 nmol/mL) who had never undergone TRT], hypogonadal [KS patients with low T levels (<12 nmol/mL) who had never undergone TRT], and TRT [KS patients who were receiving TRT and had normalized T levels (>12 nmol/mL)] (Fig. 1).

2.2. Metabolic status

Body weight was measured to the nearest 0.1 kg and height to the nearest 0.5 cm. Body mass index (BMI) was calculated and the waist circumference measured. Blood pressure was measured by sphygmomanometer using an appropriate cuff after at least 15 min of rest.

Total body fat and TrBF were assessed by whole body dual energy X-ray absorptiometry (DEXA) scans performed on a Hologic osteodensitometer (QDR Discovery Acclaim, Hologic Inc., Waltham, MA). Fasting blood samples were taken in the morning from all study participants for evaluation of lipid and glucose metabolism (fasting glucose, total cholesterol, HDL and LDL cholesterol, triglycerides).

2.3. Hormone assays

Fasting blood samples were taken in the morning for measurement of reproductive hormone [FSH, LH, total testosterone (T), estradiol (E2)

Table 2
Main parameters of the KS study population and the two control subgroups divided by BMI (NOb: BMI <30 kg/m^2) and OB: BMI ≥30 kg/m^2.

	NOb	KS	OB
N	39	221	38
Age (years)	34.2 ± 10	34.2 ± 12	33.8 ± 11
Testosterone (nmol/L)	21.1 ± 2.2	16.2 ± 8.8*	17.2 ± 3.2
BMI (kg/m^2)	25.3 ± 3.1	24.2 ± 5.3	35.6 ± 3.8*
SPB (mm Hg)	115.6 ± 8.8	114.8 ± 16.3§	126.3 ± 9.8*
DBP (mm Hg)	78.3 ± 5.3	74.5 ± 10.8§	84.4 ± 4.7*
Total cholesterol (mg/dl)	139 ± 27.4	180 ± 30.4*§	217 ± 23.5*
HDL cholesterol (mg/dl)	45.9 ± 8.8	47.6 ± 12.1	43 ± 6.5
LDL cholesterol (mg/dl)	88.8 ± 23.3	110.6 ± 29.6*§	151.2 ± 17.9*
Triglycerides (mg/dl)	69.9 ± 14	104.3 ± 65*	126.9 ± 23*

* $P < 0.05$ vs. NOb.
§ $P < 0.05$ vs. OB.

Table 3
Main metabolic parameters and blood testosterone levels of the KS patients divided into the three subgroups.

	Eugonadal	Hypogonadal	TRT
N	69	40	75
Age (years)	28.8 ± 10	35.8 ± 13	38.3 ± 11
Testosterone (nmol/L)	16.4 ± 3.9	5.5 ± 3.4*§	20.3 ± 9.8*
BMI (kg/m^2)	22.6 ± 4.8	25.7 ± 6.5*	25.1 ± 5.2*
SBP (mm Hg)	110.5 ± 17.3	119.8 ± 12.7*	117.2 ± 15.5*
DBP (mm Hg)	72.6 ± 11.2	77.4 ± 9.2	75.2 ± 10.9
Total cholesterol (mg/dl)	172.6 ± 27.3	183.1 ± 31	185.3 ± 31.8*
HDL cholesterol (mg/dl)	49.5 ± 11.3	48.5 ± 8.2	45.6 ± 13.7
LDL cholesterol (mg/dl)	102.7 ± 26.7	114 ± 29.2	151.2 ± 17.9*
Triglycerides (mg/dl)	92.2 ± 53	112.4 ± 63.7	111.8 ± 73.8

* $P < 0.05$ vs. eugonadal.
§ $P < 0.05$ vs. TRT.

and sex hormone binding globulin (SHBG)] concentrations. FSH, LH, T, E2 and SHBG were analyzed by chemiluminescent microparticle immunoassay (CMIA, Architect System, Abbott Laboratories, IL, USA), with limits of detection (LOD) of 0.05 IU/mL, 0.07 IU/mL, 0.28 nmol/L, ≤10 pg/mL, and ≤0.1 nmol/L respectively. Intra-assay and inter-assay coefficients of variations for our laboratory were: 3.6% and 5.4% at 3.2 IU/mL (FSH); 3.8 and 5.5% at 4.1 IU/mL (LH); 2.1 and 3.6% at 10.08 nmol/L (T); 4.2 and 6,1% at 36 pg/mL (E2); 5.65 and 9.54% at 28.8 nmol/L (SHBG). Normal reference ranges for adulthood were: 1.38–9.58 IU/mL for FSH; 1.79–8.17 IU/mL for LH; 10.40–38.20 nmol/L for T; 11–44 pg/mL for E2; and 11.2–78.1 nmol/L for SHBG [27].

2.4. Echocardiography

All echocardiography examinations were performed in random order by the same experienced echocardiologist, who was blinded from all clinical data. An ultrasound system equipped with a 2.5 MHz transducer (Esaote MyLab™40) was used to complete M-mode and two-dimensional, Doppler and tissue Doppler echocardiography using standardized techniques in accordance with American Society of Echocardiography guidelines. EFT was measured on the free wall of the right ventricle from both parasternal long-axis and short-axis views at end-diastole in three cardiac cycles. The maximum value at any site was measured, and the average value was considered.

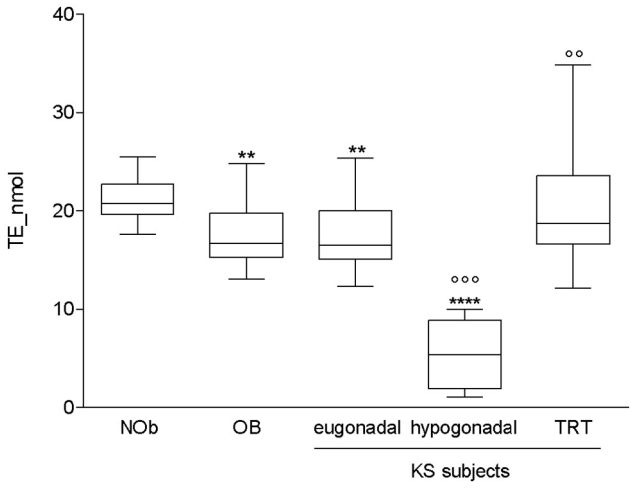

Fig. 1. Testosterone serum levels (nmol/mL) in the five subgroups: eugonadal, hypogonadal and TRT KS patients and NOb and OB control subjects. [*] indicates significant difference ($P < 0.05$) vs. NOb; [°] indicates significant difference ($P < 0.05$) vs. OB.

2.5. Statistics

Statistical analysis was performed using Prism for Windows version 4 (GraphPad software, Inc.). Data in box plots are reported as median, minimum and maximum values (whisker). Unpaired two-tailed *t*-tests or, when appropriate, non-parametric tests (Mann Whitney test) were used for comparisons of the data after testing for normal distribution. To detect determinants of EFT and TrBF, univariate linear regression analyses were performed. Multivariate models, corrected for age and including variables showing significant correlation in univariate analysis, were carried out in all subjects to investigate the association between EFT or TrBF and metabolic parameters. A 95% confidence level ($P < 0.05$) was used as the cut-off for statistical significance.

3. Results

The EFT of the KS population as a whole was significantly higher than in the NOb subgroup ($P < 0.05$) and lower than in the OB subgroup ($P < 0.05$) (Fig. 2).

When divided by subgroup, the BMI of the eugonadal KS patients was significantly lower than that of all other patient and control subgroups (Fig. 3A). There was no difference in BMI between the hypogonadal and TRT patient subgroups.

When divided by subgroup, EFT was significantly higher in the hypogonadal KS subgroup than in NOb or eugonadal KS patients ($P < 0.05$), but there was no difference in comparison with OB. In eugonadal KS patients, EFT was similar to both TRT patients and NOb, and lower than in OB and hypogonadal KS patients ($P < 0.05$). EFT was higher in TRT patients than in NOb, but lower than in OB ($P < 0.05$) (Fig. 3B).

TrBF was higher in hypogonadal KS patients than in NOb and eugonadal and TRT KS subjects ($P < 0.05$), but there was no difference in comparison with OB (Fig. 3C).

We then decided to investigate any correlations between EFT and T. No statistically significant correlation between EFT and T was found in the KS population, but an inverse correlation was observed in euploid controls ($P < 0.0001$, $R^2 = 0.36$). In both KS and control populations, there was a significant direct correlation between EFT and BMI, as expected, ($P < 0.0001$, $R^2 = 0.20$; $P < 0.0001$ and $R^2 = 0.53$ respectively), and between EFT and TrBF ($P < 0.0001$ and $R^2 = 0.27$; $P < 0.0001$ and $R^2 = 0.39$ respectively). A direct and strong correlation between EFT and TrBF was also observed in the untreated KS population ($P < 0.0001$ and $R^2 = 0.71$) (Fig. 4). Additional correlations were found between EFT and the parameters age and triglycerides ($P < 0.0001$ and $P < 0.005$ respectively). In multiple regression analyses including the most

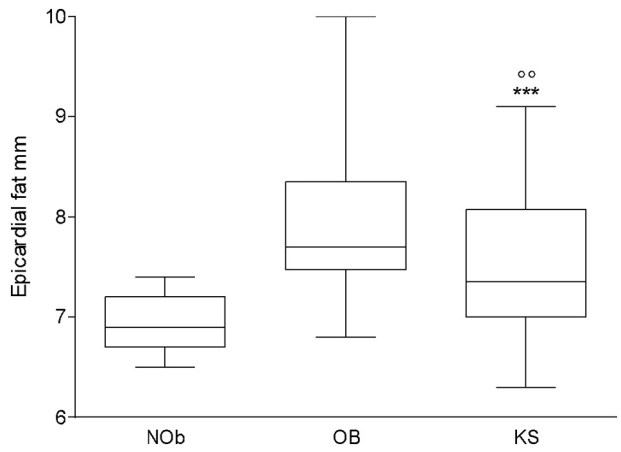

Fig. 2. Comparison of EFT (millimeters) in KS subjects and the two control groups: NOb and OB. The symbol [*] indicates significant difference ($P < 0.05$) vs. NOb; the symbol [°] indicates significant difference ($P < 0.05$) vs. OB.

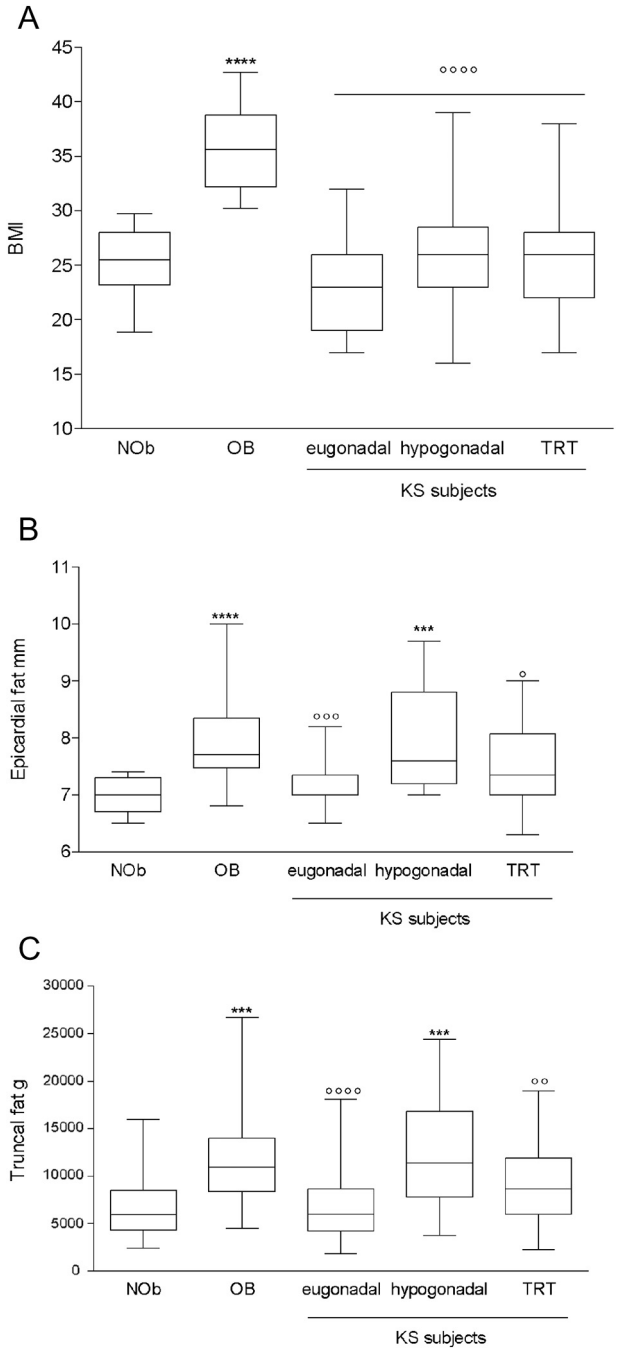

Fig. 3. BMI (kg/m^2) (A), EFT (millimeters) (B) and TBF (g) (C) in the population subgroups: eugonadal (KS males who had never undergone TRT and had normal serum testosterone levels), hypogonadal (KS patients who had never undergone TRT and had low serum testosterone levels), TRT (KS patients under TRT who had reached normal testosterone serum levels), NOb, and OB controls. [*] indicates significant difference ($P < 0.05$) vs. NOb; [°] indicates significant difference ($P < 0.05$) vs. OB.

important variables (age, BMI, T, triglycerides, presence or absence of supernumerary X chromosome), we found that apart from BMI and age, the presence of KS was the only significant contributor to EFT and TrBF (Table 4).

4. Discussion

Epicardial fat is a measurable risk factor, as it can be detected and assessed with standard imaging techniques. Given this measurability and its fast response to fat-targeting drugs, epicardial fat is considered a novel diagnostic marker and therapeutic target in cardiometabolic

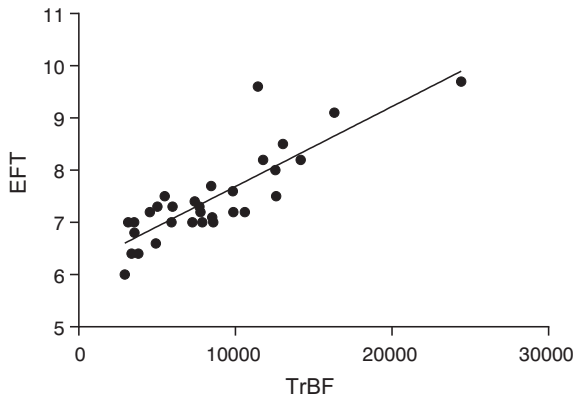

Fig. 4. Correlation between TrBF (millimeters) and EFT (g) in the KS population. Only patients who had never undergone TRT (hypogonadal and eugonadal KS) have been considered (R^2 0.71; $P < 0.0001$).

diseases, including diabetes and obesity [28]. This study evaluated, for the first time, epicardial adipose tissue in KS men with the 47, XXY karyotype.

KS men have an almost five-fold higher incidence of metabolic disorders [29]. It is possible that the increased risk of metabolic syndrome and insulin resistance in XXY males is secondary to increased abdominal obesity, which may derive from hypogonadism and low T levels [9–11,30]. Although TRT was shown to be associated with an improvement in body composition and an increase in muscle mass in 46, XY men [15] and can improve metabolic parameters such as blood glucose and insulin resistance in KS patients [7], no data have been published about the correlation between EFT and T values.

In this cross-sectional cohort study, we observed a significant difference in EFT in the different subgroups. EFT was similar in hypogonadal KS patients and obese controls, even though the BMI of the hypogonadal KS patients was significantly lower than OB group. In fact, regardless of their BMI, the EFT of hypogonadal KS patients was greater than in the non-obese control subjects. These patients also had significantly lower T levels than the OB controls, confirming the limited or lack of effect of T serum levels on epicardial adipose tissue accumulation. Eugonadal KS patients showed no difference in EFT compared to NOb or the TRT subgroup.

Table 4
Multivariate analysis of EFT and TrBF. The parameters showing a significant correlation with EFT and TrBF (age, testosterone, BMI and triglycerides) were used to produce four models, each with an increasing number of parameters. Two different multivariate analyses were performed to study EFT and TrBF.

	EFT			TrBF		
	P value	r^2	*P* value	*P* value	r^2	*P* value
Model 1		0.15	<0.0001		0.15	<0.0001
Age	<0.0001			<0.0005		
Testosterone	<0.05			<0.05		
Model 2		0.31			0.22	<0.0005
Age	<0.01			<0.001		
Testosterone	NS			<0.05		
BMI	<0.0001			<0.005		
Model 3		0.33			0.30	<0.0001
Age	<0.005			<0.0005		
Testosterone	NS			0.0153		
BMI	<0.0001			<0.005		
Triglycerides	NS			NS		
Model 4		0.62			0.59	<0.0001
Age	<0.05			<0.05		
Testosterone	NS			NS		
BMI	<0.0001			<0.0005		
Triglycerides	NS			NS		
KS	<0.0001			<0.0001		

TrBF in eugonadal KS patients was similar to that in NOb. In contrast, despite the normalized T values in the TRT subgroup, their TrBF was higher than seen in NOb, although lower than in OB. Given that this is a cross-sectional study we cannot reach any conclusions about the role of TRT on TrBF, but it could be speculated that TRT does not affect TrBF but can reduce epicardial adipose tissue accumulation in KS men. To further investigate this, we are currently conducting a longitudinal study to analyze the effect of TRT on epicardial adipose tissue.

The expected correlation between serum T levels and EFT in KS patients was not confirmed, but a significant inverse correlation was observed in the controls. However, the present study did show the expected strong correlation between EFT and BMI and between EFT and TrBF in both KS patients and controls. The correlation between EFT and TrBF was even stronger and more representative in KS who had never undergone TRT. This observation confirms the known role of EFT as an important marker of visceral fat and metabolic disorders. As expected, EFT was directly correlated with serum glucose, total cholesterol, LDL and triglyceride levels in the KS population.

US-measured epicardial fat is also associated with liver steatosis and surrogate markers of fatty liver [17]. In the present study, 64% of the KS patients who had undergone abdominal ultrasound were found to have fatty liver, with liver steatosis found in 92.3% of hypogonadal men, 46.4% of eugonadal men and 66.7% of the TRT group. This confirmed the role of liver steatosis as a marker of visceral body fat, with a distribution similar to that observed for EFT and TrBF in KS subjects.

Neither testosterone nor triglycerides seemed to play a significant role in adipose tissue distribution. Multivariate analyses showed that the major determinants of both EFT and TrBF were BMI and KS itself. These findings further emphasize the importance of the supernumerary X chromosome in the manifestation of metabolic disorders associated with KS. Moreover, in line with literature evidence that EFT could be a marker of cardiac and metabolic dysfunction in subjects with metabolic syndrome, this study showed for the first time that it may have a similar role in subjects with KS. Given the strong correlation between TrBF and EFT, the latter could be conveniently measured in KS subjects to allow routine follow-up without the need for any extra radiation exposure or invasive procedures. The great similarity in the body compositions of hypogonadal KS and obese euploid eugonadal men should guide physicians towards testosterone treatment and encourage them to start TRT as soon as needed.

5. Conclusions

This study investigated epicardial adipose tissue in KS men. Ectopic fat was accumulated in the hearts of hypogonadal KS men in a similar way to that seen in obese age-matched euploid subjects. A strong correlation between EFT and TrBF was described in both KS and control population. The presence of the supernumerary X chromosome, together with BMI, appeared to be one of the strongest determinants of both EFT and TrBF, independent of testosterone levels.

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Compliance with ethical standards

Conflicts of interest

The authors declare that they have no conflict of interest.

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