Endocrine Connections Publish Ahead of Print, published on October 22, 2013 as doi:10.1530/EC-13-0067

The macrophage low-grade inflammation marker sCD163 is modulated by exogenous sex steroids

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Abbreviated title: sCD163 and sex steroids

Keywords: sCD163, CD163, Klinefelter syndrome, Turner syndrome, low-grade inflammation, chronic inflammation.

Word count (abstract): 257

Word count (entire manuscript): 3.078

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Disclosure statement: The authors have nothing to disclose.

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Abstract

Objectives: Soluble CD163 (sCD163) is a novel marker linked to states of low grade inflammation such as diabetes, obesity, liver disease and atherosclerosis, all prevalent in subjects with Turner and Klinefelter Syndromes. We aimed to assess the levels of sCD163 and the regulation of sCD163 in regards to treatment with sex hormone therapy in males with and without Klinefelter Syndrome and females with and without Turner Syndrom. **Patients and participants:** Males with Klinefelter Syndrome (n=70) and age-matched controls (n=71) participating in a cross-sectional study and 12 healthy males from an experimental hypogonadism study. Females with Turner Syndrome (n=8) and healthy age- matched controls (n=8) participating in a randomized cross-over trial. **Intervention:** Treatment with sex steroids. **Results**: Males with Klinefelter Syndrome had higher levels of sCD163 compared with controls (1.75 (0.47-6.90) and 1.36 (0.77-3.11) respectively, $p<0.001$) and the levels correlated to plasma testosterone (r -0.31, $p<0.01$), BMI (r 0.42, $p<0.001$) and HOMA IR (r 0.46, $p<0.001$).Treatment with testosterone did not significantly lower sCD163. Females with Turner Syndrome not receiving hormone replacement therapy had higher levels of sCD163 than their age-matched healthy controls (1.38±0.44 vs. 0.91±0.40, $p=0.04$). Hormone replacement therapy and oral contraceptive therapy decreased sCD163 in Turner Syndrome by 22% (1.07±0.30) and in controls by 39% (0.55±0.36), with significance in both groups ($p=0.01$ and $p=0.04$). **Conclusions**: Levels of sCD163 correlates with endogenous testosterone in Klinefelter Syndrome and are higher in Klinefelter syndrome subjects compared to controls, but treatment did not significantly lower levels. Both endogenous and exogenous estradiol in Turner Syndrome was associated with lower levels of sCD163.

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Introduction

The CD163 molecule, expressed by cells of the monocyte lineage, particularly the macrophages, is part of a scavenger system with a high affinity for the hemoglobin-haptoglobin complex. It contains 9 scavenger-receptor cysteine-rich (SRCR) domains that are located on the extracellular side of the cell membrane (1). CD163 is expressed at different levels in different organs and in response to varying local chemical signals (2). One of its main and well described functions is the removal of plasma hemoglobin through endocytosis of the very high-affinity complex hemoglobin-haptoglobin thus preventing the oxidative stress from free hemoglobin by the release of the free iron, bilirubin and carbon monoxide. Glucocorticoids and anti-inflammatory cytokines like IL-6 and IL-10 induce increased CD163 expression thus assigning CD163 anti-inflammatory effects. Its pro-inflammatory effects are seen by the down regulation of CD163 by inflammatory cytokines such as TNF-α (tumor necrosis factor) and GM-CSF (granulocyte–macrophage colony-stimulating factor) (3,4). A soluble form of CD163 (sCD163) is formed by proteolytic cleavage of the extracellular part of the protein and shed into circulation (1,5). The function of sCD163 is not clear; however a role in the elimination of Staphylococcus aureus has recently been described (6) in addition to findings of anti-inflammatory effects through inhibition on T lymphocyte activation and proliferation (3,4).

Recently increased plasma levels of sCD163 have been linked to states of low grade inflammation such as diabetes, obesity, liver disease, and atherosclerosis (7,8,9,10,11,12,13), underscoring the important role of macrophages in initiating and propagating these conditions. Previously, we and others have shown that both females with Turner syndrome (TS) and males with Klinefelter syndrome (KS) show evidence of low-grade inflammation (14,15,16) and this substantiates the increased frequency of type 2 diabetes, disease of the circulatory system including valvular heart disease, pulmonary embolism, but excluding ischaemic heart disease in KS, and aortic valve disease, hypertension, aortic aneurysm and ischaemic heart disease in TS (17,18,19,20,21,22).

The changes seen in TS and KS is not fully understood and it is not known if the changes are entirely due to hypogonadism and most patients will require treatment with sex hormones for long periods of their lives. The low grade inflammation seen in KS and TS are influenced by the low levels of sex hormones (23,24) and 17β-estradiol and testosterone are known to modulate the inflammatory state of macrophages(25,26). An experiment of testosterone and estradiol cultured Hofbauer cells (fetal macrophages) found no influence on CD163 expression (27).

The aim of the present study was firstly to investigate the regulation of the sCD163 in TS and KS and its relation to other markers of low grade inflammation and secondly to investigate whether treatment with sex hormones, i.e. estrogen-progestin and testosterone, would impact circulating levels of sCD163.

To that end we studied samples drawn from one study with KS, one with TS (28,29,30), and one experimental study of acute male hypogonadism. We hypothesized that hypogonadism in both sexes would be accompanied by raised levels of sCD163 and that this is associated with other markers of low-grade inflammation.

Materials and methods

Klinefelter syndrome cross sectional study

Seventy subjects with KS were recruited from fertility and endocrine outpatient clinics and compared to healthy age-matched controls (n=71) recruited from at the University of Aarhus and the Blood Bank of Aarhus University Hospital. Inclusion and exclusion criteria as previously described (30). Half of the KS were receiving testosterone supplementation with testosterone injections (n=20), testosterone undecanoate (n=14) and mesterolon (n=1) whereas the other half did not receive treatment. All received oral and written information concerning the study prior to giving written informed consent. Data regarding glucose and bone mineral metabolism has previously been presented (14,31). The protocol was approved by the Aarhus County Ethical Scientific Committee (no. 20010155) and the Danish Data Protection Agency.

Experimental male hypogonadism study

Twelve healthy, non-smoking male volunteers participated in this study. All volunteers displayed

normal primary and secondary sex characteristics and none of them used medication or had a positive family history of diabetes. Men who were planning to participate in competitive sport events during the subsequent year were not included. All had levels of testosterone 18.6 (8.3-32.9) nmol/L as well as luteinizing hormone (LH) 4.8 (1.7-8.1) IU/L and follicle stimulating hormone (FSH) 3.2 (1.2-6.6) IU/L within the normal range. Other details on the study group have been described previously (32). In short, hypogonadism was achieved by subcutaneous injection of GnRH agonist (7.5 mg leuprorelide, Eligard®, Astellas Pharma, Switzerland) before 3 of 4 trial sessions. Thus, hypogonadal trial days were preceded by at least 7-10 days of castrate levels of testosterone, designed to achieve stable changes in their metabolic state. The four study arms were three hypogonadal arms with either a 50-mg or 150-mg of testosterone gel or a placebo gel applied along with an eugonadal control arm. Trial sessions included baseline measurements and testosterone treatment. Insulin sensitivity was assessed using the hyperinsulinemic euglycemic clamp technique. All volunteers received oral and written information concerning the study prior to giving written and informed consent. The protocol was approved by the Aarhus County Ethical Scientific Committee (no. M-20070046), registered at ClinicalTrials.gov (NCT-00613288), and performed in accordance with the Helsinki Declaration II.

Turner syndrome treatment study

A total of 8 subjects with TS were compared to 8 age-matched healthy controls. The design was a randomized cross-over study. Both groups underwent a 2 month wash-out period from hormone replacement therapy (HRT) and oral contraceptive therapy, respectively. Subjects were examined at the end of each 2-month period. The treatment consisted of 2 mg 17β-estradiol per day (day 1-22), 1 mg norethisterone day (day 13-22) and 1 mg 17β-estradiol per day (day 23-28) (Trisekvens®, Novo Nordisk A/S, Copenhagen, Denmark) for the subjects with TS. The controls received combined contraceptive pills. Other details on the study group have been described previously (30,33). All subjects received oral and written information concerning the study prior to giving written informed consent. The protocol was approved by the Aarhus County Ethical Scientific Committee (no. 1996/3561).

Assays

The plasma concentration of sCD163 was determined in duplicate in samples that had been frozen at -20°C by an in-house sandwich enzyme-linked immunosorbent assay using a BEP-2000 ELISA-analyser (Dade Behring, Deerfield, IL, USA) essentially as previously described (29). The duration of storage was 2-8 years. Briefly, rabbit anti-CD163 (2 mg/L) was coated onto microtitre wells and plates transferred to a BEP-2000 enzyme-linked immunosorbent assay (ELISA)-analyzer (Dade Behring, Eschborn, Germany). Samples (diluted 1:101) were added in duplicates and incubated for 1.5 h at 37 °C. Monoclonal anti-CD163 (GHI/61, 3 µg/mL) was then added for 1 h at 37 °C, followed by incubation for 1 h at 37 °C with horseradish peroxidase-labelled goat antimouse antibodies (0.125 µg/mL; Dako, Glostrup, Denmark). The plates were developed with tetramethylbenzidine (TMB) substrate solution (Kem-En-Tec, Taastrup, Denmark). The assay was calibrated using serum traceable to purified human CD163, with the lowest calibrator being 6.25 µg/L. The inter-assay coefficient of variation on control samples included on each plate (15 runs) was 3.6 % at 1.90 mg/L and 4.4 % at 3.61 mg/L.

Adiponectin was determined by use of an in-house timeresolved immonoflourometric assay(TR-IFMA) as described (34). Leptin was determined by a commercial radioimmunoassay (Linco, St.Louis, MO). Total insulin-like growth factor 1 (IGF-1) was measured by use of in-house noncompetitive, timeresolved immonoflourometric assays after acid-ethanol extraction of serum as described (35). C-reactive protein (CRP) was measured by an ultrasensitive assay (Diagnostic Products, Los Angeles, CA). Androgens, estrogens, sex hormone-binding globulin (SHBG), luteinising hormone (LH) and follicle stimulating hormone (FSH) were analyzed as described (31).

Statistical analysis

We used paired samples t-test and otherwise independent samples t-test, as appropriate. Correspondingly, Mann–Whitney U test or Wilcoxon signed rank test was used in analysis of nonparametric data. We used Pearson's or Spearman's coefficient of correlation as appropriate. In the Klinefelter syndrome cross sectional study we compared untreated males with KS and controls, and then untreated vs. testosterone treated males with KS. Because sCD163 correlated to a host of variables we went on to perform stepwise multivariate regression analysis in order to evaluate

the impact of independent variables on the dependent variable, sCD163, in the KS group and healthy subject group separately and combined. Significance level for entering and for removal of variables from the model was $p<0.05$ and $p>0.10$, respectively. We had no valid information on timing of the last intramuscular injection of testosterone among KS. Likewise, untreated females with TS were compared with untreated controls and then untreated and treated females with TS were compared. All results are shown as mean ± SD or median ± range as appropriate. Statistical analysis of data was carried out using the SPSS software (SPSS Inc., Chicago, IL, USA), version 20 for Windows. For the Experimental Hypogonadism Study, statistical comparisons for groups over time were analyzed by repeated-measures ANOVA. One-way ANOVA was used to analyze base line data (t=-120) and at the end of the clamp period (t=360). We considered three statistical models of relevance: The full “4” arm model, the “3” arm model consisting of the hypogonadal arms only, thereby assessing the acute intervention with the placebo arm as the functional “control” arm, and lastly the “2” arm model comparing sustained hypogonadism to the eugonadal state.

Results

Klinefelter syndrome cross sectional study

Characteristics for KS and controls are presented in Table 1. As previously described the control subjects were significantly leaner than the subjects with KS, had a lower body fat percentage (BF) and a higher lean body mass (LBM). KS had a significantly higher prevalence of diabetes and their metabolic profile was altogether less favourable with regards to insulin resistance, obesity, diabetes and hypertension (14). The level of sCD163 was significantly higher in the KS group by 29 % (Figure 1). There was no significant difference in levels between untreated and treated KS subjects (2.00 (0.83-4.03) vs 1.72 (0.47-6.90) mg/L, p=0.18). We therefore analysed the correlation between sCD163 and other variables in the whole KS group (treated and untreated). In KS subjects, sCD163 correlated with BMI (r=0.360, p<0.001), lean body mass (r=-0.310, p=0.02) and other body measures such as total body fat, fat on trunk, waist/hip-ratio or waist circumference. Furthermore there was correlation with testosterone (r=-0.306, p<0.01) and other androgens, HOMA IR (r=0.456, p<0.001), VO_2max (r=-0.262, p=0.04), IGF-1 (r=-0.297, p=0.01) and CRP (r= 0.302, p=0.01). In controls sCD163 correlated with BMI (r=0.360, p =0.002), lean body mass (r= -0.283, p=0.02) and similar body measures. Among controls the testosterone (r=-0.306, p=0.01) also correlated with sCD163 along with VO_2max (r=-0.27, p=0.02), but not with HOMA IR, CRP, dehydrotestosterone and IGF-1 (table 2, figure 2). Subsequent multiple linear regression analyses in the combined population of KS and controls was done with sCD163 as the dependent variable. BMI, testosterone and status (KS or control) were the only independent variables (R=0.579, p<0.0001). Thus, these variables explain about 35 % of the variation in sCD163, with prominent differences between KS and controls.

Experimental Hypogonadism study

To investigate the direct effect of testosterone on sCD163 levels, we included data from an experimental hypogonadism study in healthy male volunteers. Baseline characteristics and levels of testosterone have been presented before (36). As expected, no differences in parameters reflecting body composition were seen during short term hypogonadism. Likewise, the

concentrations of triglycerides (TG), VLDL-TG, free fatty acids, cortisol, insulin, glucose and glucose infusion rates were comparable during both basal and clamp periods in all statistical models (data not shown). Short term hypogonadism did not affect levels of sCD163 (P=NS, basal period, one-way ANOVA), nor did testosterone treatment in any model affect levels at the end of a 3 hour hyperinsulinemic euglycemic clamp (P=NS, one-way ANOVA and repeated measures ANOVA, respectively).

Turner syndrome treatment study

Baseline characteristics for TS and controls are presented in Table 3. As previously described, controls and TS had similar BMI, fat mass and fat-free mass as well as similar HOMA IR. TS had lower levels of estradiol and testosterone and higher levels of FSH and LH (37). In the untreated state sCD163 was significantly higher among TS subjects compared to controls (Figure 3). Treatment with HRT or contraceptive pills (with ethinyl estradiol as the active ingredient) respectively significantly lowered levels of sCD163 in both TS and controls (Figure 3). Our 17-beta-estradiol assay does not pick up ethinyl estradiol hence suppressed levels of estradiol is seen in the controls receiving OCT. CRP was significantly higher in the TS subjects in the untreated situation than in the in the control group. During active treatment CRP increased in controls due to oral contraceptive therapy while it did not change significantly in TS subjects. There was no correlation between sCD163 and any of the other measured variables in either TS or controls.

Discussion

The present study shows that sCD163, as a macrophage-based marker of chronic low-grade inflammation, is elevated in both Turner and Klinefelter syndrome. In addition, we show that sCD163 is influenced by both endogenous testosterone, and endogenous and exogenous estradiol and norethisterone, which is a novel finding.

Whether presence of low-grade inflammation has any clinical significance in disorders of chromosomal anomalies has yet to be investigated, but we know from previous studies that the risk of type 2 diabetes and cardiovascular diseases are markedly increased in both TS and KS (21,22,38,39). Markers of low-grade inflammation have been shown to predict mortality and morbidity in various diseases (40,41,42,43,44,45). Males with KS suffer from low-grade inflammation compared to age-and sex -matched controls, as shown here by elevated sCD163, but also shown previously with other markers of low-grade inflammation like CRP (14,46). We saw no difference between KS treated with testosterone and untreated KS in sCD163 levels. In multiple linear regression analyses, we could show that BMI, but also status (i.e. KS or control) remained independent contributors of the level of sCD163 in the combined study group. We and others have previously shown that abdominal adiposity, insulin resistance and outright type 2 diabetes are frequent occurrences in KS (47,14,39,48) and low grade inflammation in subjects with KS could likely attribute to their metabolic phenotype which in turn, at least in part, is due to their relative hypogonadism. Our data do not indicate that treatment with testosterone reverses the low grade inflammation, as evident by sCD163, because levels were similar in the treated and untreated KS groups. Levels of sCD163 is higher amongst obese subjects compared to lean subjects and positively correlated to various other unfavourable metabolic features (9) and indeed, though in lesser part, to levels of adipocytokines. The failure to demonstrate significant efficacy in lowering sCD163 levels by testosterone treatment in our KS subjects may be due to the duration of treatment. It also has to be kept in mind that the study was not randomized or otherwise designed to prove efficacy of testosterone treatment, but was merely observational.

Previously, we have shown that high-sensitive CRP and TNF-α is higher in TS compared with controls and speculated that this is due to a chronic condition with low-grade inflammation

(49,50,15). We have now shown that this is accompanied by increased sCD163 (Turner syndrome treatment study), and a striking down regulating effect on sCD163 of both HRT in TS and contraceptive pills in controls (-22% and -39%, respectively). Interestingly, the down regulatory effect on sCD163 of HRT in TS and oral contraceptive therapy in controls is contrary to the effects on CRP-levels (increased by oral contraceptive therapy in controls and unchanged in TS). This finding in controls is in concert with others (51) and suggests that different estrogens, i.e. natural 17-β estradiol and synthetic ethinyl estradiol, do indeed have differential effects on markers of inflammation. Because controls received oral combination contraceptive pills and though TS subjects were sampled in the "follicular phase" any impact on our result from the added progestins is unknown. Estradiol has in macrophages been shown to act anti-inflammatory and suppress TNF-α through suppression of NF-κB activation (52), but results are not uniform. In a study of women with hyperinsulinemic androgen excess, oral contraceptive therapy lead to an increase in CD163 gene expression contrary to our findings in a hypogonadal model (53). The CD163 expression correlated to unfavourable metabolic features (e.g. increase in visceral fat). In the other study arm metformin and flutamide treatment induced a more metabolically favourable profile and lead to a lowering of CD163 gene expression. This has also been established in vitro where metformin was also shown to downregulate CD163 (54). The reason for the discordant results is probably explained by the distinct metabolic differences between TS and women with PCOS. In a study using isolated human-monocyte-derived macrophages, estradiol did not affect the production of TNF-α or other cytokines, and CRP was affected variably depending on the pertinent level of LDL-cholesterol, with high levels of LDL leading to larger production of CRP, while low levels of LDL led to diminished CRP (55). Moreover, sCD163 is specifically produced by macrophages whereas CRP is predominantly produced by hepatocytes. Our results may therefore be interpreted as a specific effect of estrogens on macrophage activity, which does influence e.g. IL-6 mediated hepatic CRP expression. Whether this effect is mediated through changes in metabolic features like BMI and insulin resistance, associated with low grade inflammation themselves (56), cannot be established by the current data and TS subjects do indeed have higher BMI, albeit not significantly in our study, than controls and this is not normalised by the short term treatment with HRT. While the short treatment duration in this study did not result in changes in body composition measures

such as BMI, it is entirely possible that the observed effects on sCD163 is mediated through regulation of adipokines by 17β-estradiol/progestin. Pro-inflammatory cytokines upregulate 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in adipose tissue and higher levels have indeed been associated with the metabolic syndrome (57) also found prevalent in both Klinefelter and Turner Syndromes (24,49). Thus at least at a localized level increased glucocorticoid action might contribute to the higher levels of sCD163 (3) as found in our study via mechanisms not yet understood. While glucocorticoids are potent inducers of increments in CD163 expression this does not necessarily increase levels of sCD163 in plasma, but when accompanied by an inflammatory stimulus it gives rise to increased cleavage activity of a disintegrin and metalloproteinase 17 known as ADAM17 and TNF-α converting enzyme (TACE) resulting in increased shedding of sCD163 from the cell surface of the glucocorticoid acitivated machrophages (58). Neither estradiol nor testosterone gave rise to increased expression of CD163 in an in vitro experiment , pointing out this intangible association between CD163 expression and the soluble form of CD163 (27).

In conclusion, we have shown that the level of sCD163 is influenced by both endogenous and exogenous sex hormones in different states of sex hormone deficiency. As a macrophage-based marker of chronic low-grade inflammation, sCD163 is elevated in both Turner and Klinefelter syndrome and correlate with indices of body composition and markers of insulin resistance. Estrogen-progestin treatment significantly decreases sCD163 in contrast to CRP. The different mechanisms of origin and activation of sCD163 and CRP might prove useful in clinical settings of different aetiologies, but warrants further targeted research to establish any firm conclusions. At present, it is not evident whether low-grade inflammation is a result of or a consequence of co-existing disease states (46) or may have both protective and harmful effects. Likewise, the association between sex hormones and the immune system needs further research to provide knowledge in a clinical setting.

Acknowledgements: The Klinefelter study was supported by the Aase and Einar Danielsen Foundation, and the Danish Diabetes Association. The experimental hypogonadism study was supported by grants from the Danish Medical Research Council and an unconditional research

grant from Ipsen Pharma. The Turner syndrome study was supported by grants from the Danish Ministry for Science Technology and Innovation, the Danish Heart Foundation, Novo Nordisk, Aase og Ejnar Danielsens Foundation, Korning Foundation, Hede Nielsens Foundation, Eva og Henry Frænkels Minde Foundation and Snedkermester Sophus Jacobsen og hustru Astrid Jacobsens Foundation. Lab technician Kirsten Bank Petersen is acknowledged for excellent technical assistance. This study was supported by grants from and the Danish Council for Strategic Research (TRAIN 10-092797, HJM).

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Table 1. Data on Klinefelter syndrome persons and controls from the Klinefelter syndrome cross sectional study, regarding inflammation markers, body composition, hormones and insulin sensitivity.

					P-value		
	Controls	**KS**	**U-KS**	**T-KS**	**Controls vs. KS***	**Controls vs. U-KS***	**U-KS vs. T-KS***
N	71	70	35	35			
Age (years)	36.4 (19.2-68.0)	35.5 (19.0-66.2)	35.0 (19.0-66.2)	38.7 (19.3-62.3)			
sCD163 (mg/L)	1.36 (0.77-3.11)	1.75 (0.47-6.90)	2.00 (0.83-4.03)	1.72 (0.47-6.90)	<0.001§	<0.001§	0.181§
Testosterone (nmol/L)	21.8 (10.6-55.5)	12.77 (0.8-72.2)	12.68 (0.8-37.3)	14.04 (1.9-72.2)	<0.001	<0.001	0.192
Estradiol (pmol/L)	81.0 (40-210)	86.0 (40-290)	77 (40-140)	89 (44-290)	0.24	0.819	0.041
BMI (kg/m²)	24.9 (19.0-36.9)	26.9 (18.1-60.6)	27.3 (20.0-60.6)	25.1 (18.1-54.7)	0.046	0.008	0.369
LBM (kg)	78.4 ±6.4	70.4 ±8.7	68.4 ±7.3	72.7 ±9.8	<0.001†	<0.001†	0.062†
BF (%)	18.9 ±6.8	26.7 ±9.2	28.7 ±7.6	24.3 ±10.4	<0.001†	<0.001†	0.069†
VO_2max (mL $O_2 \cdot kg^{-1} \cdot min^{-1}$)	43.5 (24.0-73.3)	29.9 (14.6-57.3)	29.9 (14.6-50.1)	29.9 (14.9-57.3)	<0.001§	<0.001§	0.943§
CRP (mg/L)	0.11 (0.02-1.99)	0.19 (0.02-2.74)	0.21 (0.03-2.74)	0.17 (0.02-1.83)	0.001	0.001	0.103
HOMA IR (%)	1.2 (0.3-5.7)	2.1 (0.04-21.3)	2.3 (0.5-10.0)	1.8 (0.4-21.3)	<0.001§	<0.001§	0.976§
Adiponectin	4.21 (2.3-9.1)	3.7 (1.4-13.6)	3.5 (1.4-9.7)	4.5 (1.6-13.6)	0.53§	0.125§	0.126§
Leptin (ng/L)	3.1 (1-17)	11.0 (2-116)	14.0 (2-116)	8.4 (2-75)	<0.001	<0.001	0.081

Data are mean ±SD or median (total range). § Student's *t*-test with ln-transformed data. * Mann-Whitney U rank sum test. † Student's *t*-test. KS, Klinefelter syndrome; U-KS, untreated-KS; T-KS, treated-KS; BMI, body mass index; LBM, lean body mass; BF, body fat; VO_2max, maximal oxygen uptake; CRP, C-reactive protein; HOMA IR, Homeostasis Model of Assessment Insulin Resistance.

Table 2 Data on Klinefelter syndrome persons and controls from the Klinefelter syndrome cross sectional study, regarding correlation analysis between sCD163 and markers of body composition, hormones, insulin sensitivity and others.

	Controls	**P-value**	**KS**	**P-value**
N	71		70	
sCD163 (ln)	1.00		1.00	
Testosterone (ln)	-0.31	<0.01	-0.31	<0.01
BMI	0.36	0.002	0.42	<0.001
Waist/hip-ratio	0.33	0.006	0.30	0.01
Fat on trunk	0.21	0.08	0.42	0.001
VO_2 max	-0.27	0.02	-0.26	0.04
HOMA IR (ln)	0.03	0.82	0.46	<0.001
IGF-1 (ln)	-0.19	0.12	-0.30	0.01
Adiponectin (ln)	0.004	0.98	-0.15	0.22
Leptin (ln)	0.29	0.014	0.45	<0.001
Insulin (ln)	0.03	0.82	0.46	<0.001
Cholesterol, total	0.16	0.18	0.08	0.51
Cholesterol, HDL (ln)	-0.10	0.40	-0.25	0.04

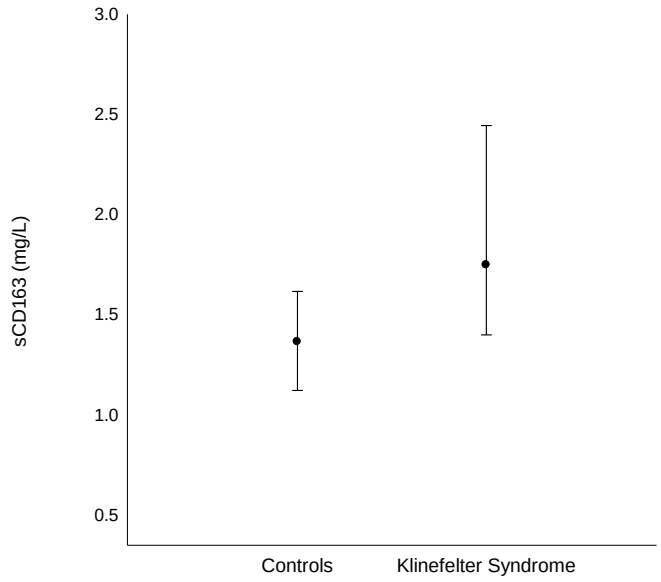
Data are Pearsons correlation coefficient (P-value). KS, Klinefelter syndrome; BMI, body mass index; LBM, lean body mass; BF, body fat; VO2max, maximal oxygen uptake; HOMA IR, Homeostasis Model of Assessment Insulin Resistance; IGF-1, insulin-like growth factor -1.

Table 3. Data on Turner syndrome subjects and controls from the Turner syndrome treatment study, regarding inflammation markers, BMI, sex hormones and insulin sensitivity.

			P-value			P-value
	Controls	**TS**	**Controls vs. TS††**	**Controls +OCT**	**TS +HRT**	**TS vs. TS +HRT†**
N	8	8				
Age (years)	28.5 ±4.2	29.1 ±5.8				
sCD163 (mg/L)	0.91 ±0.40	1.38 ±0.44	0.04	0.55 ±0.36	1.07 ±0.30	0.01
BMI (kg/m^2)	21.9 (20-30)	25.9 (22-31)	0.07**	21.7 (19.9-29.9)	25.3 (22.6-33.0)	0.67*
Estradiol (pmol/L)	320 (20-610)	110 (90-150)	0.01**	115 (70-240)	220 (110-1280)	0.02*
Testosterone (nmol/L)	2.18 ±0.59	1.41 ±0.77	0.04	1.35 ±0.41	1.20 ±0.75	0.32
CRP (mg/L)	0.09 (0.04-0.16)	1.03 (0.15-2.04)	0.01	0.24 (0.12-1.53)	0.73 (0.23-2.50)	0.52§
HOMA IR	0.56 ±0.28	0.71 ±0.24	0.28	0.60 ±0.21	0.80 ±0.43	0.36

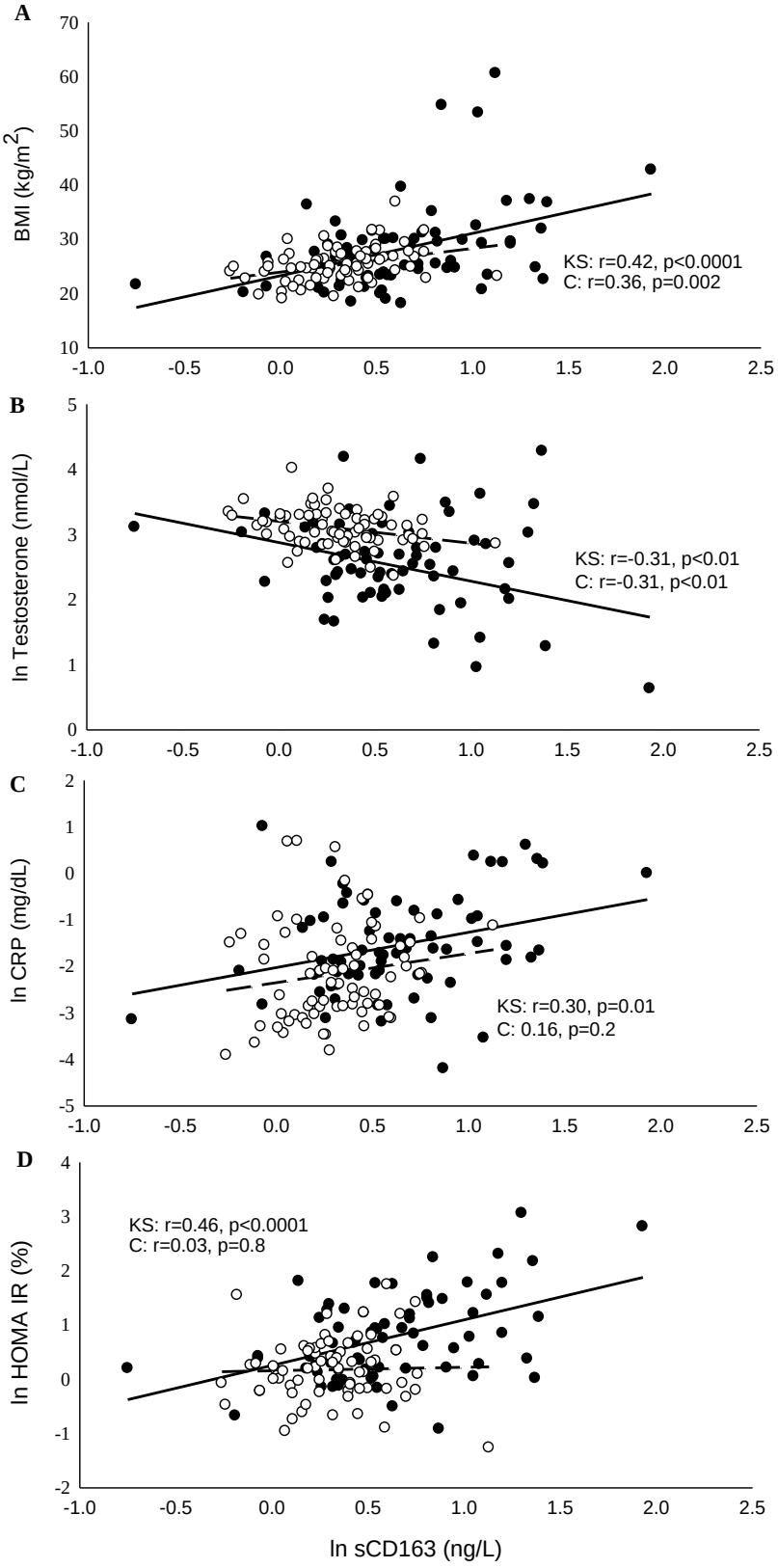
Data are mean±SD or median (total range). † Paired samples *t*-test. †† Independent samples *t*-test * Mann-Whitney U rank sum test, dependent samples. **Mann-Whitney U rank sum test, independent samples. § Paired samples *t*-test with ln-transformed data. TS, Turner syndrome; OCT, oral contraceptive therapy; HRT, hormone replacement therapy; BMI: body mass index; CRP: C-reactive protein; HOMA IR: Homeostasis Model of Assessment Insulin Resistance.

Figure 1 Klinefelter syndrome cross sectional study.



Data presented are median with 25th and 75th percentiles. P-value is p<0.001.

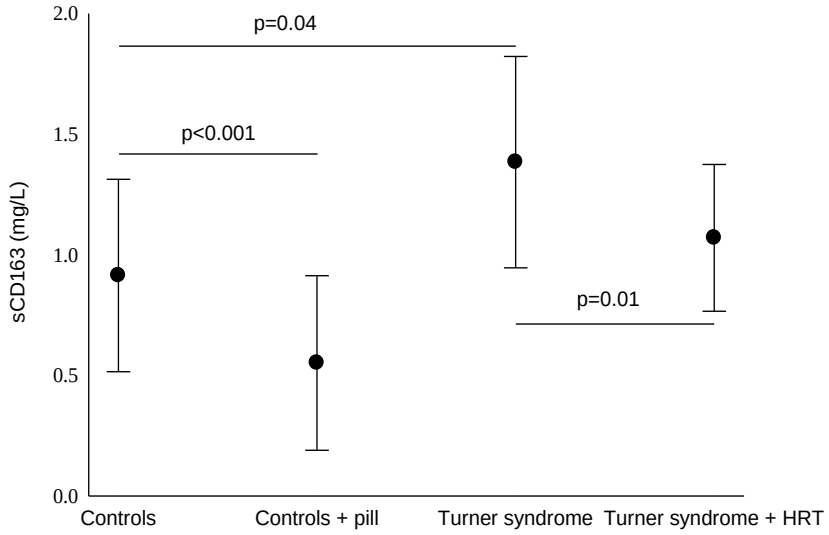
Figure 2 Klinefelter syndrome cross sectional study



sCD163 plotted against different variables. Open circles (○) represent controls and filled circles(●)

represents Klinefelter syndrome (KS) subjects. Regression lines are inserted – solid lines indicate KS and broken lines indicate controls. P-values and r-values are inserted in all figures. A: BMI. sCD163 is positively and uniformly associated to BMI in both the KS and control group. B: Testosterone correlates to sCD163 in the two groups C: CRP correlates to sCD163 in both groups. D: Insulin sensitivity (HOMA IR) correlates to sCD163 in KS subject, but not in controls.

Figure 3 Turner Syndrome treatment study



Data presented as mean±SD. P-values are indicated in the figure.