CLINICAL STUDY

Serum sex steroids measured in middle-aged European and African–Caribbean men by gas chromatography–mass spectrometry

Frank Giton^{1,2,*}, Jean Fiet^{2,*}, Jean-Nicolas Cornu³, Olivier Cussenot^{3,4}, Alain Bélanger⁵, Saïk Urien⁶, Alejandro Oliva⁷, Pascal Blanchet^{8,9} and Luc Multigner⁹

¹AP-HP CIB GHU Sud, Hôpital Henri Mondor, Creteil, France, ²INSERM U955 Eq07, Faculty of Medicine, Centre de Recherches Chirurgicales, 8 Rue du Général Sarrail, 94010 Creteil cedex, France, ³Department of Urology, Tenon Hospital, AP-HP, University Paris VI, Paris, France, ⁴CeRePP, Tenon Hospital, AP-HP, Paris, France, ⁵Laboratory of Molecular Endocrinology and Oncology, Centre de Recherche du Centre Hospitalier Universitaire de Québec (CR-CHUQ) and Faculty of Medicine, Laval University, Québec City, Québec, Canada, ⁶Clinical Research Unit, Tarnier Hospital, Paris, France, ⁷Unidad de Andrología, Hospital Italiano, Rosario, Argentina, ⁸Department of Urology, CHU Pointe-à-Pitre, Guadeloupe, French West Indies and ⁹INSERM U625, Pointe-à-Pitre, Guadeloupe, French West Indies

(Correspondence should be addressed to J Fiet; Email: fiet@univ-paris12.fr)

*(F Giton and J Fiet contributed equally to this work)

Abstract

Background: Differences in circulating steroid hormone levels have been hypothesized to explain ethnic differences in steroid-related diseases. The aim of this study was to determine the serum levels of a wide panel of steroid hormones, both androgens and estrogens, in healthy middle-aged African–Caribbean and European men.

Design and methods: Serum steroid hormone levels were determined in men participating in a systematic public health study funded by the French National Health Insurance system. Blood was collected in the morning from 304 healthy African–Caribbean and European men aged between 40 and 69 years. Serum steroids were measured by mass spectrometry–gas chromatography, except for DHEAS and sex hormone-binding globulin, which were determined by RIA. Data were analyzed in 10-year age intervals by analysis of covariance, with adjustment for age, body mass index, waist-to-hip ratio, tobacco and alcohol consumption, and season of sampling.

Results: Compared with Europeans, African–Caribbean men presented significantly higher serum levels of measured bioavailable testosterone, 4-androstenedione (4-dione), and estrone (E1) regardless of the age group, of 5-androstenediol (5-diol) in those aged 40–49 and 50–59 years, and of testosterone (TT) and dihydrotestosterone in those aged 40–49 years. In contrast, European men aged 40–69 years showed significantly higher serum levels of DHEA and DHEAS.

Conclusions: Significant differences in serum steroid hormone levels were observed in middle-aged African–Caribbean and European men. Whether such differences could contribute to ethnic differences in disease risk in adult men remains to be investigated. Some steroids, such as bioavailable TT, 4-dione, 5-diol, and E1, deserve particular attention.

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Introduction

Ethnic differences in the incidence of cardiovascular disease (1), osteoporosis (2), bone fractures (3), and prostate cancer (4) in men, have been hypothesized to be related to lifelong differences in androgen or estrogen exposure.

Several studies comparing blood androgen and estrogen concentrations in various ethnic groups, particularly between African-American and Caucasian men (5–17), have resulted in conflicting results. Moreover, because of

differences in steroid hormone assay methods and/or the broad age range of patients included in the reference studies, it is difficult to achieve a generally consistent analysis of these papers. To overcome these limitations, we focused on two healthy, middle-aged (40–69 years) male populations of African and Caucasian origin. In these two ethnic groups, we examined and compared a wide panel of blood androgens and estrogens at 10-year age intervals. Steroid hormones were measured by gas chromatography–mass spectrometry (GC–MS), a method considered to be the gold standard for steroid hormone assay (18).

Materials and methods

Population

Subjects were recruited between 2005 and 2006 among 40- to 69-year-old men participating in a free yearly systematic health-screening program open to general public funded by the French National Health Insurance system. Each year, a random population sample selected in accordance with the sex and age distribution of the general population is invited to participate in the program. Caribbean subjects of African descent (African–Caribbeans) were recruited in Les Abymes, the biggest city in Guadeloupe (French West Indies), a Caribbean archipelago in which most inhabitants are of African descent (19). European subjects of Caucasian descent (Europeans) were recruited in Tours, the biggest city in the Indre-et-Loire Province (mainland France) (20). Subjects were selected according to the birthplaces of their parents as follows: any Caribbean island the population of which is predominantly of African descent for African–Caribbean subjects and Western Europe for European subjects.

Information was obtained from participants regarding demographic characteristics, anthropometrics (weight, height, waist, and hip measurements), lifestyle (past or current tobacco and alcohol consumption), medical records, and medication use. A blood sample was drawn from each participant between 0800 and 1000 h, after a recommended overnight fast. Inclusion criteria were as follows: body mass index (BMI) < 30, no history of medical disorder (including cancer), standard biochemical and hematological blood parameters in the normal range, no hormone treatments or drugs known to influence the hypothalamic/pituitary/gonadal and adrenal axes, a

normal digital rectal examination, a total plasma prostate-specific antigen concentration lower than the 75th percentile, when plotted against the age group for African-American men without clinical evidence of prostate cancer for subjects of African origin (21), and lower than 1.5 ng/ml for subjects of European origin.

Five hundred consecutively enlisted men aged 40–69 years of age in each location were invited to participate in the study. The acceptance rate was around 90% in both cities. After exclusion of men who did not fulfill the inclusion criteria or who had provided a blood sample insufficient to carry out all hormonal assays, 304 African–Caribbeans and 325 Europeans were finally enrolled in the study.

The study was approved by both the Guadeloupe and Tours University Hospital Ethics Committees for studies involving human subjects. Each participant provided written informed consent.

Laboratory procedures

After clotting, serum was separated and kept at -30°C . Samples were blinded and analyzed in a randomized manner. Three control serum samples used, as quality control plus a serum of very low concentrations were included in each run.

DHEA, $\Delta 4$ -androstenedione (4-dione), $\Delta 5$ -androstenediol (5-diol), testosterone (TT), dihydrotestosterone (DHT), estrone (E1), estradiol (E_2), and E1 sulfate (E1-S) were assayed simultaneously by MS coupled with GC on 1000 μl serum (20, 22, 23). Briefly, deuterated steroid internal standards (CDN Isotopes, Inc., Point-Claire, Quebec, Canada) were added to all serum samples and extracted with 1-chlorobutane. The organic extracts

Table 1 Analytical control validation and normal serum values for young Caucasians. The data for LLOQ, low, middle, and high QC samples are presented as means of 32 runs (intra- and inter-assay coefficients of variation (%)).

Assay method/ analytes	TIA/IS (amu)	Det. range (nmol/l)	LLOQ (nmol/l)	Low QC (nmol/l)	Middle QC (nmol/l)	High QC (nmol/l)	Normal Caucasian values ^a
GC–MS^b							
DHEA	482/485	0.64–28.09	0.64 (5.4–6.1)	2.60 (3.5–4.7)	6.90 (2.6–2.8)	17.58 (1.4–2.0)	25.69 (12.60–43.65)
4-Dione	461/468	0.17–14.14	0.17 (6.2–10.8)	1.68 (3.7–4.9)	3.53 (3.1–4.1)	7.05 (2.4–3.8)	4.71 (2.59–7.54)
5-Diol	678/683 ^a	0.37–13.95	0.37 (7.0–11.0)	1.06 (4.0–4.4)	2.55 (3.7–4.2)	5.10 (2.7–3.2)	6.78 (3.58–11.37)
TT	482/485	0.17–28.09	0.17 (4.8–10.1)	1.66 (2.2–3.1)	8.53 (2.1–2.5)	20.60 (1.0–1.7)	17.72 (10.88–26.14)
E1	464/468	29.1 ^c –2996 ^c	29.1 ^c (7.4–9.5)	53.3 ^c (4.3–4.7)	288.1 ^c (3.1–3.3)	735.6 ^c (2.6–3.0)	113.9 ^c (59.2–163.3)
E ₂	660/664	7.7 ^c –1487 ^c	7.7 ^c (8.9–10.5)	36.0 ^c (3.5–4.1)	176.2 ^c (2.6–3.0)	728.0 ^c (1.5–1.7)	114.5 ^c (81.9–151.2)
E1-S	464/468 ^d	0.13–16.11	0.13 (8.7–11.0)	1.34 (4.0–4.4)	4.00 (3.1–3.4)	7.98 (2.8–2.9)	2.55 (1.21–4.28)
DHT	484/487	0.07–13.95	0.07 (7.8–9.0)	0.65 (3.0–3.1)	1.65 (2.0–2.3)	5.13 (1.7–1.7)	2.03 (1.14–3.16)
ASP (20) + GC–MS							
BT				1.01 (3.2–3.5)	3.74 (4.2–4.3)	8.98 (1.4–1.5)	6.93 (4.37–10.34)
RIA							
SHBG		6.0–164.0		16.0 (2.0–2.3)	41.9 (2.9–3.3)	68.4 (1.8–2.3)	27.4 (14.3–41.7)
DHEAS		0.23 ^e –23.05 ^e			1.4 ^e (2.2–3.5)		8.07 ^e (4.28–11.55)

ASP, ammonium sulfate precipitation; IS, internal standard (3β -diol- d_3 (5α -androstane- 3β , 17β -diol- d_3 , 683 amu); LLOQ, lower limit of quantification; QC, quality control; TIA, target ions analyte; Det., detection.

^a20–39 years ($n=147$; mean (10th to 90th percentiles)).

^bAccuracy for eight steroids measured by GC–MS in human samples: -3.8 to $+3.4\%$ ($n=6$).

^cMeans of the QC serums are expressed in nmol/l except E_2 and E1 in pmol/l.

^dAfter solvolysis.

^eMeans of the QC serums are expressed in nmol/l except DHEAS in $\mu\text{mol/l}$.

were purified on conditioned high-purity silica LC-Si SPE columns (Varian, Les Ulis, France). DHEA, 5-diol, TT, DHT, E1, and E₂ were derivatized with pentafluorobenzoyl chloride (103772, Aldrich) and 4-dione with pentafluorobenzylhydroxylamine (P4190, Aldrich). The final extracts were reconstituted in isooctane, and then transferred into conical vials for injection into the GC system (6890N, Agilent Technologies, Massy, France), using a 50% phenylmethylpolysiloxane VF-17MS capillary column (20 m×0.15 mm, internal diameter, 0.15 µm film thickness; Varian). An HP5973 (Agilent Technologies) quadrupole mass spectrometer equipped with a chemical ionization

source and operating in single ion monitoring mode was used for detection. E1-S was measured as E1 after acid solvolysis (23) (Table 1).

In Table 1, we report the molecular mass of the derivatized steroids (deuterated and corresponding non-deuterated steroids) assayed by GC-MS. We also report the means and intra- and inter-assay coefficients of variation (CV) of four quality control sera: one with very low concentrations of the assayed steroids to determine the lower limit of quantification and three with increasing concentration levels (low, middle, and high).

Bioavailable testosterone (BT) was measured by addition of purified tritiated TT to the serum samples, incubating and then precipitating the sex hormone-binding globulin (SHBG)-bound TT with saturated ammonium sulfate (20, 24). After centrifugation, we deduced the percentage of SHBG-unbound tritiated TT (BT%), and multiplying by total TT concentration, we obtained the serum BT concentration. DHEAS was measured by a radioimmuno-logical method (IM 0729, Beckman Coulter, Marseille, France). Plasma SHBG levels were measured by a radioimmunological method (Schering RIACT, Gif-sur-Yvette, France). The results of BT, DHEAS, and SHBG quality control are reported in Table 1.

Statistical analysis

We normalized the distributions by applying a square root for SHBG or a log10 transformation for hormones. We used the Kolmogorov-Smirnov test to ensure normality. The transformed values were converted back to normal values for presentation of result.

We used the χ^2 test for categorized variables and the unpaired *t*-test for continuous variables. Mean blood hormone levels were compared between the ethnic groups by analysis of covariance. The following covariates were considered potentially confounding factors: age (years), BMI (kg/m²), waist-to-hip ratio, alcohol (never vs ever) and tobacco consumption (never vs ever), and season of blood collection. For all subjects and for each 10-year group, covariates that differed ($P < 0.1$) between the two ethnic groups and that were associated ($P < 0.1$) with hormone or protein levels were included in the model. By a backward elimination procedure, covariates associated with $P > 0.1$ were removed. Age was maintained in the final model regardless of significance level. All analyses were carried out using the Statview Software package (SAS Institute, Inc., Cary, NC, USA). All tests were two sided, and *P* values < 0.05 were considered to be statistically significant.

Results

Baseline characteristics of African-Caribbean and European men stratified in 10-year intervals are reported in Table 2. Mean concentrations of each steroid

Table 2 Baseline characteristics of the subjects.

Characteristics	African-Caribbeans	Europeans	<i>P</i>
Age (years; mean (s.d.))			
All ages	57.0 (7.7)	53.9 (7.9)	<0.0001
40 to <50 yoa	46.0 (3.0)	44.0 (2.9)	<0.0001
50 to <60 yoa	55.0 (2.7)	54.8 (3.0)	0.52
60 to <70 yoa	64.8 (3.0)	62.9 (2.3)	<0.0001
Body mass index (kg/m ² ; mean (s.d.))			
All ages	24.6 (2.8)	25.1 (2.4)	0.02
40 to <50 yoa	24.7 (2.7)	24.6 (2.4)	0.69
50 to <60 yoa	24.7 (3.1)	25.0 (2.3)	0.32
60 to <70 yoa	24.5 (2.7)	25.8 (2.4)	0.0003
Waist-to-hip ratio (mean (s.d.))			
All ages	0.90 (0.08)	0.91 (0.06)	0.006
40 to <50 yoa	0.86 (0.07)	0.89 (0.05)	0.002
50 to <60 yoa	0.90 (0.08)	0.92 (0.06)	0.18
60 to <70 yoa	0.91 (0.08)	0.93 (0.05)	0.03
Former or current smokers, <i>n</i> (%)			
All ages	116 (38)	159 (49)	0.006
40 to <50 yoa	21 (33)	55 (55)	0.007
50 to <60 yoa	52 (43)	64 (50)	0.22
60 to <70 yoa	43 (36)	40 (41)	0.48
Former or current drinkers, <i>n</i> (%)			
All ages	249 (84)	296 (91)	0.006
40 to <50 yoa	48 (79)	86 (86)	0.23
50 to <60 yoa	109 (90)	117 (92)	0.57
60 to <70 yoa	92 (80)	93 (95)	0.001
Season of blood collection, <i>n</i> (%)			
All ages			
Spring	72 (24)	34 (11)	<0.0001
Summer	71 (23)	13 (4)	
Autumn	80 (26)	81 (25)	
Winter	81 (27)	197 (61)	
40 to <50 yoa			
Spring	32 (51)	3 (3)	<0.0001
Summer	7 (11)	0 (0)	
Autumn	1 (2)	0 (0)	
Winter	23 (37)	97 (97)	
50 to <60 yoa			
Spring	21 (17)	5 (4)	<0.0001
Summer	36 (30)	0 (0)	
Autumn	41 (34)	52 (41)	
Winter	24 (30)	70 (55)	
60 to <70 yoa			
Spring	19 (16)	26 (27)	0.11
Summer	28 (24)	13 (13)	
Autumn	38 (32)	29 (26)	
Winter	34 (29)	30 (31)	

yoa, years of age.

hormone tested are presented in Table 3 as well as the 10th and 90th percentiles. Adjusted mean concentrations are presented in Table 4 as well as the 95% CI. After adjustment, the African–Caribbean men showed significantly higher concentrations of some androgens

Table 3 Mean hormone concentrations (back-transformed mean (10th to 90th percentile)) in African–Caribbean and European men of all ages and by 10-year age interval.

Hormones	African–Caribbeans	Europeans	P
Testosterone (nmol/l)			
All ages	18.27 (11.37, 29.37)	16.82 (11.30, 24.65)	0.002
40 to <50 yoa	20.42 (12.03, 30.20)	17.58 (12.03, 27.22)	0.007
50 to <60 yoa	17.65 (11.20, 26.91)	16.71 (11.20, 24.55)	0.22
60 to <70 yoa	17.82 (11.20, 29.51)	16.30 (10.96, 23.44)	0.03
BT (nmol/l)			
All ages	6.38 (2.84, 7.56)	4.61 (4.16, 9.99)	<0.0001
40 to <50 yoa	7.87 (6.00, 11.48)	5.55 (3.36, 8.29)	<0.0001
50 to <60 yoa	6.45 (4.16, 9.99)	4.40 (2.88, 6.73)	<0.0001
60 to <70 yoa	5.65 (3.78, 8.29)	4.02 (2.50, 6.14)	<0.0001
DHT (nmol/l)			
All ages	1.96 (0.96, 3.68)	1.79 (1.03, 2.93)	0.009
40 to <50 yoa	2.07 (1.07, 3.75)	1.65 (0.86, 2.86)	0.002
50 to <60 yoa	1.89 (0.90, 3.27)	1.93 (1.17, 3.00)	0.58
60 to <70 yoa	2.00 (0.96, 4.20)	1.72 (1.03, 2.93)	0.03
DHEA (nmol/l)			
All ages	9.99 (5.06, 18.20)	11.48 (5.34, 24.93)	0.002
40 to <50 yoa	12.69 (6.93, 22.36)	16.89 (8.11, 30.17)	0.0004
50 to <60 yoa	10.58 (5.37, 19.07)	10.85 (5.62, 20.87)	0.74
60 to <70 yoa	8.22 (4.47, 15.85)	8.32 (3.81, 16.19)	0.90
DHEAS (μmol/l)			
All ages	2.89 (1.25, 5.99)	3.57 (1.54, 7.37)	<0.0001
40 to <50 yoa	3.25 (1.58, 6.89)	5.19 (2.74, 9.30)	<0.0001
50 to <60 yoa	2.68 (1.54, 6.29)	3.34 (1.85, 6.00)	0.27
60 to <70 yoa	2.52 (1.17, 5.48)	2.65 (1.31, 5.23)	0.58
4-Dione (nmol/l)			
All ages	4.02 (2.44, 6.77)	3.32 (1.92, 5.66)	<0.0001
40 to <50 yoa	4.82 (3.11, 7.65)	4.12 (2.37, 6.35)	0.007
50 to <60 yoa	3.95 (2.41, 6.08)	3.28 (1.99, 5.52)	0.001
60 to <70 yoa	3.74 (2.30, 6.22)	2.65 (1.68, 2.90)	<0.0001
5-Diol (nmol/l)			
All ages	4.99 (2.10, 10.40)	4.27 (2.27, 7.33)	0.0003
40 to <50 yoa	7.06 (3.44, 12.78)	4.82 (3.06, 7.54)	<0.0001
50 to <60 yoa	5.30 (2.65, 10.16)	4.24 (2.27, 8.06)	0.0007
60 to <70 yoa	3.82 (1.72, 8.64)	3.79 (1.86, 6.40)	0.82
E ₂ (pmol/l)			
All ages	109.8 (72.7, 164.8)	105.0 (77.8, 145.4)	0.07
40 to <50 yoa	112.1 (58.2, 139.5)	103.7 (65.3, 118.8)	0.09
50 to <60 yoa	106.4 (54.3, 139.5)	107.3 (63.8, 130.2)	0.77
60 to <70 yoa	112.4 (58.2, 136.4)	103.9 (56.8, 124.4)	0.05
E1 (pmol/l)			
All ages	150.2 (100.2, 235.6)	101.7 (58.1, 172.0)	<0.0001
40 to <50 yoa	136.5 (95.0, 198.6)	93.1 (53.4, 140.6)	<0.0001
50 to <60 yoa	150.7 (97.3, 233.3)	106.9 (64.2, 181.1)	<0.0001
60 to <70 yoa	159.2 (101.8, 250.0)	104.2 (58.6, 173.0)	<0.0001
E1-S (nmol/l)			
All ages	1.61 (0.75, 3.65)	1.64 (0.83, 3.14)	0.92
40 to <50 yoa	1.40 (0.73, 3.28)	1.48 (0.75, 2.55)	0.55
50 to <60 yoa	1.69 (0.75, 3.79)	1.69 (0.89, 3.22)	0.88
60 to <70 yoa	1.69 (0.62, 3.30)	1.72 (0.91, 3.30)	0.88
SHBG (nmol/l)			
All ages	34.9 (16.7, 58.3)	34.2 (20.2, 52.4)	0.25
40 to <50 yoa	30.6 (14.6, 54.4)	29.4 (15.9, 46.7)	0.68
50 to <60 yoa	32.0 (15.1, 54.0)	35.5 (20.3, 58.8)	0.05
60 to <70 yoa	40.6 (20.1, 71.4)	35.1 (21.3, 58.4)	0.01

yoa, years of age.

(TT, BT, 4-dione, and 5-diol) and of one estrogen (E1), and lower concentrations of the androgen conjugate (DHEAS), compared with the European men. When stratified by age groups in 10-year intervals, higher levels of BT, 4-dione, and E1 were significant, regardless of the age group. However, after stratification, in the African–Caribbeans, significant higher TT and DHT and significantly lower DHEA and DHEAS were observed only in the 40- to 49-year interval and higher 5-diol levels in the 40–49 and 50–59 year intervals. Slightly higher E₂ levels, but no significantly higher adjusted E₂ levels, were found in the African–Caribbeans. No differences were observed between the ethnic groups for SHBG and E1S, regardless of the age group.

Discussion

In this article, we report the blood levels of major steroid hormones involved in the steroid metabolic pathway, mostly measured by GC–MS, in two populations of non-obese, healthy, middle-aged men of two ethnic backgrounds: African–Caribbean and European. We compared these hormone levels both overall and in 10-year age intervals. In the broad 40- to 69-year age interval, we found TT levels to be higher in African–Caribbeans than in Europeans. Similarly, higher TT levels were reported in five of the 12 studies comparing African-Americans and white Americans (Table 5). When we analyzed the data in 10-year age intervals, the TT level remained statistically higher in African–Caribbean only in the 40- to 49-year age interval. As the results comparing African–Caribbeans and Europeans were age dependent, it could be important to extend this comparison to groups of these populations aged between 20 and 40 years. Indeed, significantly higher TT levels were reported in rather narrow age intervals of populations of young African-Americans than in young white American students 25–36 and 18–24 years old (Table 5).

As was the case for TT, we also found higher DHT in African–Caribbeans than in Europeans in the age range of 40–49 years, but not in the other age ranges or in the wide 40- to 69-year interval. Of the six studies known to report DHT in African-Americans and in white Americans, three reported significantly higher DHT in African-Americans (Table 5) in broad age intervals. Moreover, all DHT assays were carried out using low-specificity immunoassays, except for the last published study (17), which used GC–MS.

We did not find SHBG concentrations to be significantly different between African–Caribbeans and Europeans regardless of the age range, which is in accordance with previous studies comparing African-Americans and white Americans (7–9, 11–14, 16).

A assayed BT, which has been described as an index of androgenicity (25), was found to be significantly higher in African–Caribbean than in European in the broad 40- to 69-year age interval and in each 10-year range.

Table 4 Adjusted mean concentrations (back-transformed mean (95% CI)) of steroid hormones in men of African–Caribbean and European origin at all ages and by 10-year age interval.

Hormones	African–Caribbeans	Europeans	Adjusted <i>P</i> ^a
Testosterone (nmol/l)			
All ages	18.27 (17.61, 19.00)	16.85 (16.23, 17.48)	0.003 ^B
40 to <50 yoa	20.42 (18.76, 22.23)	17.55 (16.40, 18.76)	0.008 ^A
50 to <60 yoa	17.65 (16.57, 18.76)	16.68 (15.71, 17.72)	0.20 ^A
60 to <70 yoa	17.58 (16.50, 18.69)	16.75 (15.64, 17.93)	0.33 ^B
BT (nmol/l)			
All ages	6.59 (6.31, 6.83)	4.47 (4.33, 4.65)	<0.0001 ^A
40 to <50 yoa	8.01 (7.35, 8.74)	5.48 (5.13, 5.86)	<0.0001 ^A
50 to <60 yoa	6.45 (6.07, 6.87)	4.40 (4.16, 4.68)	<0.0001 ^C
60 to <70 yoa	5.76 (5.41, 6.14)	3.99 (3.71, 4.26)	<0.0001 ^A
DHT (nmol/l)			
All ages	1.93 (1.83, 2.03)	1.83 (1.72, 1.89)	0.14 ^D
40 to <50 yoa	2.03 (1.79, 2.27)	1.65 (1.52, 1.83)	0.01 ^E
50 to <60 yoa	1.86 (1.72, 2.03)	1.93 (1.79, 2.10)	0.44 ^A
60 to <70 yoa	1.93 (1.76, 2.13)	1.83 (1.62, 2.03)	0.38 ^B
DHEA (nmol/l)			
All ages	10.51 (9.92, 11.13)	10.96 (10.37, 11.58)	0.29 ^A
40 to <50 yoa	13.25 (11.69, 15.01)	16.50 (14.98, 18.20)	0.01 ^C
50 to <60 yoa	10.68 (9.74, 11.69)	10.82 (9.88, 7.28)	0.84 ^A
60 to <70 yoa	8.39 (7.63, 9.26)	8.11 (7.32, 9.05)	0.68 ^F
DHEAS (μmol/l)			
All ages	3.02 (2.83, 3.21)	3.43 (3.23, 3.64)	0.004 ^C
40 to <50 yoa	3.32 (2.90, 3.80)	5.11 (4.59, 5.68)	<0.0001 ^A
50 to <60 yoa	3.12 (2.85, 3.41)	3.33 (3.04, 3.63)	0.33 ^A
60 to <70 yoa	2.62 (2.36, 2.92)	2.53 (2.25, 2.85)	0.67 ^A
4-Dione (nmol/l)			
All ages	4.12 (3.95, 4.29)	3.25 (3.11, 3.35)	<0.0001 ^B
40 to <50 yoa	4.85 (4.43, 5.34)	4.09 (3.81, 4.40)	0.006 ^A
50 to <60 yoa	3.95 (3.70, 4.22)	3.28 (3.07, 3.49)	<0.0001 ^A
60 to <70 yoa	3.74 (3.49, 4.02)	2.62 (2.41, 2.83)	<0.0001 ^A
5-Diol (nmol/l)			
All ages	5.17 (4.86, 5.48)	3.55 (3.89, 4.37)	<0.0001 ^A
40 to <50 yoa	7.13 (6.40, 7.95)	4.79 (4.41, 5.23)	<0.0001 ^A
50 to <60 yoa	3.89 (3.65, 4.17)	3.24 (3.03, 3.44)	0.0004 ^A
60 to <70 yoa	3.99 (3.62, 4.44)	3.65 (3.24, 4.10)	0.26 ^A
E ₂ (pmol/l)			
All ages	109.6 (106.1, 113.3)	105.2 (102.0, 108.6)	0.08 ^A
40 to <50 yoa	110.8 (103.4, 118.8)	104.2 (98.7, 110.1)	0.18 ^A
50 to <60 yoa	106.4 (101.0, 112.4)	107.2 (101.9, 112.8)	0.83 ^A
60 to <70 yoa	111.8 (105.9, 118.0)	104.6 (98.5, 111.0)	0.11 ^A
E1 (pmol/l)			
All ages	148.8 (142.3, 155.6)	102.7 (98.3, 107.2)	<0.0001 ^A
40 to <50 yoa	139.7 (126.4, 154.5)	91.7 (84.8, 99.2)	<0.0001 ^C
50 to <60 yoa	150.6 (140.4, 161.7)	107.1 (99.9, 114.7)	<0.0001 ^A
60 to <70 yoa	157.0 (145.8, 169.4)	104.8 (96.6, 113.8)	<0.0001 ^A
E1-S (nmol/l)			
All ages	1.64 (1.53, 1.75)	1.61 (1.53, 1.72)	0.92 ^G
40 to <50 yoa	1.40 (1.21, 1.61)	1.45 (1.29, 1.64)	0.71 ^A
50 to <60 yoa	1.69 (1.53, 1.85)	1.69 (1.53, 1.88)	0.89 ^A
60 to <70 yoa	1.72 (1.56, 1.93)	1.69 (1.50, 1.91)	0.83 ^B
SHBG (nmol/l)			
All ages	33.5 (31.9, 37.5)	34.8 (33.2, 36.4)	0.26 ^G
40 to <50 yoa	30.2 (26.7, 34.0)	29.5 (26.8, 32.4)	0.76 ^A
50 to <60 yoa	31.8 (29.4, 34.3)	35.6 (33.1, 38.2)	0.11 ^C
60 to <70 yoa	38.6 (35.7, 41.4)	37.7 (34.6, 41.0)	0.72 ^B

yoa, years of age.

^aAdjusted to ^Aage; ^Bage and body mass index; ^Cage and season; ^Dage, body mass index, and alcohol; ^Eage and waist to hip ratio; ^Fage and alcohol, ^Gage, body mass index, and waist-to-hip ratio.

In previous reports, the serum BT level was not different between African-Americans and white Americans (Table 5). The significantly higher assayed BT in African–Caribbeans is consistent with the higher 5-diol levels we found in them. 5-diol has an SHBG association constant similar to that of TT (26) and contributes to the displacement of plasma TT from binding to SHBG (20), consequently to the increase in SHBG-unbound TT and BT in African–Caribbeans.

We found higher 4-dione and 5-diol levels in African–Caribbean men compared with Europeans. Such differences were significant in each 10-year range for 4-dione, and in the 40–49 and 50–59 age intervals for 5-diol.

The 5-diol steroid, whose plasma level represents one-third of TT, deserves more study, considering its action on the estrogen receptor and on SHBG, thereby facilitating TT release and increasing androgen action. Indeed, 5-diol has a direct androgenic action, as reported (27), since it can activate the androgen receptor without being metabolized into TT, and has an indirect androgenic effect, since it increases SHBG-unbound TT and BT (20).

Contrary to other androgens, we found DHEAS to be significantly lower in the overall population of African–Caribbeans than in Europeans, as previously reported in three studies (Table 5) as well as in the 40- to 49-year age interval in our study. In our older African–Caribbeans (50–59 and 60–69 year age intervals), this difference became non-significant, though DHEAS decreased more steeply with age in the Europeans. We also found DHEA to be lower in African–Caribbeans in the 40- to 49-year age interval.

We found a slight but non-significantly higher E₂ level in African–Caribbeans than in Europeans. Previous studies have led to conflicting conclusions, some showing no significant difference, others reporting higher levels in African-Americans compared with white Americans (Table 5).

An interesting finding is the significantly higher E1 level in African–Caribbeans compared with Europeans. Mean serum E1 levels were ~150% higher in African–Caribbeans compared with Europeans regardless of the age interval investigated. These same higher E1 levels in middle-aged men were also reported in younger and older men of African descent (Table 5). Thus, E1 levels appeared to be constantly higher throughout adulthood in men of African descent compared with Caucasians. These higher E1 levels could be related to the significantly higher levels of the 4-dione precursor (28) that we observed in our African–Caribbean population regardless of the age range investigated.

Compared with previous studies (Table 5), ours has several strengths. We simultaneously measured a large number of androgens and estrogens. Except for DHEAS, we used GC–MS for our steroid hormone assays. This sensitive and specific method is currently considered the most accurate for measuring steroid hormones (18). We excluded obese subjects and those with acute or

Table 5 Mean blood steroid hormone values and methodology assays used in previous studies comparing African-American and Caucasian men.

References/ethnicity	n	Age ranges	TT (nmol/l)	BT (nmol/l)	DHT (nmol/l)	DHEA (μmol/l)	DHEAS (nmol/l)	E ₂ (pmol/l)	E1 (pmol/l)
Ross <i>et al.</i> (5)		Students	RIA					RIA	RIA
African	50		22.9					77	135.4
Caucasian	50		19.1*					71.2	116.6*
Ellys & Nyborg (6)		30–50							
African	525		2.8						
Caucasian	3654		22.1*						
Wu <i>et al.</i> (7)		> 60	RIA	ASD	RIA				
African	315		17.2	5.48	1.68				
Caucasian	411		16.3	5.3	1.54*				
Ettinger <i>et al.</i> (8)		25–36	IM	CAL		IM			
African	109		20.6	11.1		5.14			
Caucasian	114		19.1*	10.3		5.68*			
Platz <i>et al.</i> (9)		47–78	RIA		RIA			RIA	
African	43		15.9		1.24			80.4	
Caucasian	55		16.3		1.24			74.1	
Asbell <i>et al.</i> (10)		> 55	RIA		RIA				
African	12		11.4		1.13				
Caucasian	26		14.2*		1.48*				
Winter <i>et al.</i> (11)		18–24	RIA					RIA	
African	23		23.3					172.4	
Caucasian	23		18.6*					157.8	
Ukkola <i>et al.</i> (12)		17–65	RIA		RIA	RIA	RIA	RIA	
African	93		14.9		2.89	5.79	13.88	78.9	
Caucasian	202		14.5		2.47*	6.46	15.9	66.4	
Gapstur <i>et al.</i> (13)		24–41	RIA						
African	483		20.1						
Caucasian	695		19.7						
Tsai <i>et al.</i> (14)		20–55	RIA	CAL				RIA	
African	238		24	14.9				179.8	
Caucasian	412		21.2*	13.3				154.1*	
Litman <i>et al.</i> (15)		30–79	ECL	CAL	RIA	ECL			
African	531		15.7	7.5	1.65	5.35			
Caucasian	702		15	7.3	1.51	5.67*			
Rohrmann <i>et al.</i> (16) ^a		45–69	ECL					ECL	
African	103		19.5					138.7	
Caucasian	201		17.3					126.2*	
Orwoll <i>et al.</i> (17)		> 65			GC–MS			GC–MS	GC–MS
African	221				1.58			85.5	149.1
Caucasian	427				1.30*			73.7*	118.6*

CAL, calculated; ASD, assayed; IM, immunoassay without further precision; GC–MS, gas chromatography–mass spectrometry. *Significantly different between ethnic groups ($P < 0.05$).

^aRohrmann *et al.* (16) also reported data from 20 to 44 years old and 70 years and older.

chronic pathological conditions suspected to be associated with changes in serum steroid hormone levels. The time of blood sampling was carefully controlled to avoid differences related to diurnal variations, and statistical analysis took into account potential confounding factors. Moreover, we investigated our population in narrow 10-year age intervals. This enabled us to discriminate age-dependent progressive variations in serum hormone levels at ~50 years of age that result from both physiological and physical changes related to aging (29). Nevertheless, we should recognize several limits: the two populations we studied come from different geographical areas, one in the tropical Caribbean and the other in the temperate Western Europe. Therefore, the influence of certain environmental factors known to influence serum steroid levels, such as diet and physical activity, cannot be excluded.

Most of the reported previous studies considered broader age ranges, which are less discriminating. Although the season of blood collection was taken into account as a confounding factor for statistical analysis, residual confounding due to differences in accumulated sunlight exposure time between the two climates cannot be excluded. Finally, the sample sizes in our study do not reveal significant mean differences between ethnic groups <5–15%, considering the standard deviation of hormone distribution in our population study (data not shown).

Circulating steroid hormone level differences between ethnic groups may be explained by different levels of transcription or allele frequency of polymorphism of steroid hormone-related genes (30). Differences in serum steroid hormone levels have been hypothesized to explain ethnic differences in the risk of some steroid

hormone-related diseases. For instance, men of African descent are known to present a higher incidence of prostate cancer (4) and a lower prevalence of vertebral fractures compared with Caucasians (3). In addition, polymorphism (repetitions and SNPs) in relevant hormone-related genes has been associated with prostate cancer risk (31–34).

In conclusion, we have shown significantly different serum steroid hormone levels in African–Caribbeans and in Europeans, particularly higher E1, delta-4-dione, and BT in African–Caribbeans. What seems important for further study would be to include the widest possible variety of androgen and estrogen assays and to use irrefutable assay methods, such as GC–MS or LC–MS/MS. Moreover, since differences in circulating hormone levels between ethnic groups depend on age, it would be interesting to investigate in which periods of life differences in hormone levels could be etiologically relevant for specific morbid conditions.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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