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Cut-off levels for hyperandrogenemia among Samoan women: An improved methodology for deriving normative data in an obese population

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ABSTRACT

Objective: To define biochemical hyperandrogenemia (HA) among a population-based sample of reproductive-aged Samoan women, taking into consideration their high BMI levels.

Design and methods: A secondary analysis was performed among a cross-sectional sample of Samoan women aged 25–39 years (n = 494) who were part of a larger genome-wide association study (GWAS) of adiposity. Women indicating pregnancy/lactation, hysterectomy, oophorectomy, cancer treatment, or use of contraceptive injections were excluded from the study.
We analyzed the distribution of free androgen index (FAI) values to establish normative androgen data among Samoan women of reproductive age. Using the lowest tertile of body mass index (BMI), we defined HA as free androgen index (FAI) values >95th FAI percentile in that subsample. We compared the anthropometric and metabolic characteristics of women with HA to women with normal androgen levels.

Results: HA was defined as FAI > 8.5. Using this definition, 14% of women were classified as hyperandrogenemic. Women with HA had significantly higher average BMI values, abdominal circumferences, fasting triglycerides, and insulin levels as well as significantly lower adiponectin levels.

Conclusion: This study is the first to define normative androgen values among Samoan women with a quantitative assessment of the relationship between adiposity and androgen levels. The uniquely high BMI levels in the population not only provide important clinical insight into normative androgen values among Samoan women, but they also serve as references for the clinical assessment of HA, taking into consideration BMI, in other populations.

1. Introduction

The prevalence of overweight and obesity among adults in Samoa is one of the highest in the world, with a higher prevalence among women compared to men. In a 2010 survey, 91.3% of women and 80.4% of men were overweight or obese, based on Polynesian cut-offs for body mass index (BMI; BMI ≥ 26 kg/m^2) [1]. Despite the increasing burden of obesity among women in developing countries, the impact of obesity on reproductive health is not well-understood. As a result, a standardized approach to clinical diagnosis and treatment for health issues such as endocrine disorders among obese women is lacking.

Adiposity in adult women is associated with hyperandrogenemia (HA) in the literature [2,3,4,5]. HA can be established clinically or biochemically. Hirsutism, a clinical manifestation of hyperandrogenemia, can be assessed using the modified Ferriman–Gallwey (mF–G) scoring system, but assessments are often subjective and normal body hair amounts and distribution can vary by ethnicity [6]. Biochemical assessment is most conducive to standardization, but a consensus has not been reached on the methodology with which to define a specific cut-off point for HA in a given population [7]. The most commonly used method is a percentile cut-off among a pre-defined "healthy" group [8,9,10,11]. However, there is inconsistency in selecting a percentile cut-off as well as in defining what constitutes a healthy group in the literature. Using pre-determined percentile cut-offs without exploratory analyses into patterns of androgen distribution does not necessarily capture physiologically normative data [12].

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Considering the rise in overweight and obesity in women of reproductive age globally, the high prevalence of these conditions and the wide range of BMIs among Samoan women can contribute to a better understanding of associations between women's adiposity and androgen levels globally. The purpose of this report is to define hyperandrogenemia among Samoan women and establish the relationship between biochemical hyperandrogenemia and cardiometabolic risk factors.

2. Materials and methods

2.1. Study population & data collection

The data for this study were derived from a parent population-based genome wide association study (GWAS) of adiposity and cardiometabolic risk factors conducted in 2010 among Samoans. The GWAS recruited adults of Samoan descent (four Samoan grandparents), aged 24.5 to <65 years, from thirty-three villages in independent Samoa [1].

Health questionnaires, anthropometric measurements, and fasting serum samples were collected. The health questionnaire included menstrual patterns and contraceptive use, but clinical signs of hyperandrogenemia were not collected. After a minimum of ten hours fasting overnight, venous whole blood specimens were collected in vacutainers spray-coated with silica and containing polymer gel for serum separation. The specimens were separated by centrifugation in the field, and serum was stored at −40°C in Samoa. Samples were shipped in bulk on dry ice to Northwest Lipid Labs, Seattle, WA for lipid and metabolic hormone assays.

2.2. Cross-sectional sample selection

A total of 3504 participants enrolled in the parent study. For this analysis, participants who had not completed the health questionnaire were excluded (n = 7). Men (n = 1416) were excluded, and only women of reproductive age, 25–39 years, were included (n = 759). The upper age limit was deliberately conservative to avoid peri-menopausal as well as postmenopausal conditions [13]. Women with normal physiologic causes of menstrual irregularity, namely pregnancy/lactation (n = 17), hysterectomy or oophorectomy (n = 14), and cancer treatment (n = 1), were excluded. Women who did not report their menstrual cycle data (n = 10) or who lacked anthropometric measurements (n = 1) or fasting serum samples (n = 49) were also excluded. Because using exogenous hormone injections for contraception can result in irregular menstruation and altered androgen levels [14], we excluded women who indicated ever using contraceptive injections (n = 173). The final study sample size for this analysis was n = 494.

2.3. Anthropometric measures

The standard procedures for anthropometric measurements have been described by Hawley et al. [1]. Categorical definitions of BMI based upon body composition measures among Polynesians were used to define overweight (BMI 26–32 kg/m^2) and obesity (BMI > 32 kg/m^2), cut-offs equivalent to the standard World Health Organization (WHO) cut-offs of 25 and 30 kg/m^2, respectively, after taking into account the greater lean mass of Polynesians [15].

Abdominal circumference was taken at the level of the umbilicus. Due to the difficulty in locating the bony markers necessary to assess waist circumference in a population with a high prevalence of overweight and obesity, abdominal circumference was used as a substitute for waist circumference in calculating the ratios abdominal circumference-to-hip ratio (AHR) and abdominal circumference-to-height ratio (AHtR). For central obesity, the WHO cut-offs are abdominal circumference >88 cm, waist-to-hip ratio >0.85, and waist-to-height ratio >0.5 [16]. Mid-upper arm and calf circumferences were used as measures of peripheral obesity.

2.4. Cardiometabolic measures

Cholesterol, triglycerides, insulin, glucose, and adiponectin assays were undertaken by Northwest Lipid Labs in Seattle, WA, USA. The analytic methods are described by Hawley et al. [1].

The variable homeostasis model assessment-estimated insulin resistance (HOMA-IR) was calculated as: HOMA-IR = fasting plasma insulin (μU/mL) × glucose (mg/dL) / 405 [17]. Because of the general similarities in body size and adiposity of Mexican Americans and Samoans, we used, albeit cautiously, the insulin resistance cut-off of HOMA-IR >3.80 recommended among Mexican Americans by Qu et al. [18].

Type 2 diabetes was defined as either a fasting glucose level of ≥126 mg/dL or self-report of taking medication for diabetes. We excluded diabetic women (n = 21) when analyzing glucose, insulin, and HOMA-IR values in the study.

2.5. Reproductive hormones

Serum levels of total testosterone (TT) and sex hormone binding globulin (SHBG) were determined by automated chemiluminescent immunoassay (Siemens, Los Angeles, CA) at Women & Infants Hospital in Providence, RI, USA. SHBG values exceeding the maximum sensitivity range of 180 nmol/L (n = 20) were diluted and re-assayed. For TT, the lower limit of detection was 0.694 nmol/L, and values at or below this level (n = 55) were assigned 0.694 nmol/L for analysis. The inter-assay coefficients of variation for TT and SHBG were less than 8.7% and 4.1%, respectively.

The free androgen index (FAI), defined as (TT × 100) / SHBG, was calculated for each sample. FAI adjusts TT values for abnormalities in SHBG, and studies have shown that FAI is a more sensitive and reliable index for assessing HA [19]. Given the variability of the TT assays currently available, using FAI provides a better estimate of TT concentration by incorporating the more accurate results of the SHBG assays [20,21].

2.6. Statistical analyses

All metabolic and reproductive hormones were subject to a log-10 transformation prior to statistical analyses to account for the highly right-skewed nature of these distributions, and comparisons are reported as median (log standard deviation). We calculated Pearson's correlation coefficients between androgen measurements and anthropometric measurements.

In order to better quantify the association of BMI and androgens in a predominantly overweight population, FAI was stratified by equal tertiles of BMI rather than by pre-defined obesity categories.

As described above, standard biochemical cut points for defining HA are not well-defined in the literature [12,22]. We defined HA here to be FAI above the 95th FAI percentile among women in the lowest BMI tertile. The 95th percentile is an accepted approach to defining biological cut-offs, but it is important that this approach be used among a subset in the population with low BMI given the strong association of BMI and FAI [4,12]. In order to have robust statistical power, we use the 95th FAI percentile in the lowest BMI tertile (n=165) instead of in the normal BMI group (BMI < 26 kg/m^2) in which there were only n = 55 women.

Differences in metabolic parameters between androgen groups were assessed using independent samples t-test or a Chi-squared test. A pairwise exclusion was used for missing data. Statistical significance was two-tailed at the p = 0.05 level.

2.7. Ethical approval

The Brown University Institutional Review Board and the Health Research Committee of the Samoan Ministry of Health approved the parent research protocol and informed consent process.

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3. Results

The sample consisted of 494 women approximately evenly distributed in 5-year age groups between 25–39 years (Table 1). The majority of women were overweight or obese, with more than half considered obese based on Polynesian BMI classifications.

A histogram of log-10 transformed FAI values (Fig. 1) appeared symmetric and unimodal at the median of FAI = 3.4. This was confirmed via examination of a probability plot that shows the data fitted a log Gaussian distribution well (data not shown).

Age was positively correlated with SHBG measurements and negatively correlated with measurements of both TT and FAI (Table 2). SHBG was negatively correlated with all body size measures. Both TT and FAI measurements had positive associations with all body size measures, with the exception of an association between TT and AHR.

Androgen measurements were then stratified by BMI categories (Table 3). Obese women had significantly higher median TT and FAI and lower median SHBG compared to both overweight women and women with normal BMI. Overweight women had lower median SHBG and higher median FAI compared to women with normal BMI.

Fig. 2 shows androgen data stratified by tertile of BMI. Mean BMI in the three groups are 26.9, 34.0 and 41.7 kg/m^2 (n = 165, 165, and 164 observations, respectively). The 95th FAI percentile values were then calculated for each of the tertiles. From lowest to highest BMI tertile, the 95th FAI percentiles are 8.5, 16.4, and 21.5. We defined HA as FAI > 8.5 for the remainder of the analyses. Table 3 shows the 95th FAI percentile among Polynesian BMI classifications for comparison.

Using the derived cut-off of FAI > 8.5, the prevalence of HA in our population of Samoan women was 14% (69/494, 95% CI 11% to 17%). Compared to women with normal androgen levels, the mean BMI of women with HA was significantly higher (Table 4), as were weight and central and peripheral adiposity measurements. Similarly, the median values of triglycerides were significantly higher among women with HA compared to those with normal androgen values, while median adiponectin was significantly lower. Non-diabetic women with HA had higher median values of insulin and HOMA-IR compared to non-diabetic women with normal androgen values.

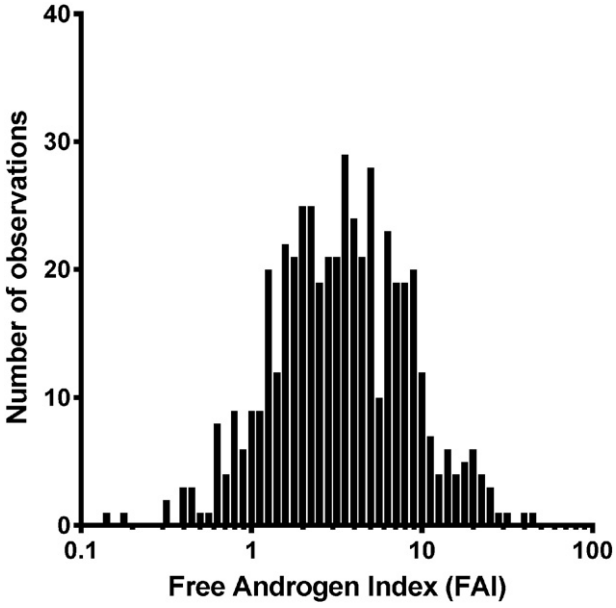

Fig. 1. Histogram of free androgen index (FAI) in 494 Samoan women. FAI results are plotted on a logarithmic x-axis with numbers of observations on the y-axis. The median FAI is 3.4 (5th and 95th FAI percentiles are 0.7 and 15.8, respectively).

4. Discussion

The results of the present study emphasize the relationship between body fat and circulating androgen levels in women and the necessity of accounting for adiposity when defining HA. We found a strong positive association between serum FAI and BMI. For this reason, we defined elevated androgen levels as FAI > 95th FAI percentile among women in the lowest BMI tertile. Using this careful criterion, 14% of Samoan women of reproductive age had biochemical HA. The women with HA had higher mean central and peripheral obesity as well as higher median levels of triglycerides and higher type 2 diabetes prevalence compared to women with normal androgen levels. Non-diabetic women with HA furthermore had higher median insulin levels and HOMA-IR compared to non-diabetic women with normal androgen levels. These findings are consistent with the key role that body fat plays in altering the secretion, transportation, metabolism, and the action of androgens, while androgens can in turn contribute to increased susceptibility to type 2 diabetes and cardiovascular disease [4,5,23,24,25]. Given the rise in average BMI and prevalence of obesity globally, other studies can use a similar approach in defining biochemical HA among their study samples. Values must be population-specific because androgen values vary across ethnicities [6]. Thus, we caution our findings may not be generalizable to other populations, as these results may be associated with Samoan genetic and life course influences on adiposity during the nutrition transition [1].

Various methods have been used to define HA in individual population studies, mostly without biological explanation and consistency [12]. Two studies, one among Chinese women and a second among Iranian women, have instead used k-means clustering to establish androgen cut-offs [12,26]. Both studies use the one-sample Kolmogorov test to find that FAI is not normally distributed in the sample. However, in order to justify the use of clustering techniques, the studies should

Table 1
Characteristics of the 494 Samoan women included in the study.

Characteristic	N	Summary of results
Mean age in years (SD)	494	32.3 (4.6)
25.0–29.9 years	179	36.2%
30.0–34.9 years	156	31.6%
35.0–39.9 years	159	32.2%
Mean BMI in kg/m^2 (SD)	494	34.2 (6.8)
Normal (<26 kg/m^2)	55	11.1%
Overweight (26–32 kg/m^2)	132	26.7%
Obese (>32 kg/m^2)	307	62.1%
Median TT in nmol/L (log SD)	494	1.6 (0.2)
Median SHBG in nmol/L (log SD)	494	45.8 (0.3)
Median FAI (log SD)	494	3.4 (0.4)
Type 2 diabetes	465	4.5%

BMI = body mass index, TT = total testosterone, SHBG = sex hormone binding globulin, FAI = free androgen index, SD = standard deviation, log SD = standard deviation computed after a logarithmic transformation.

Table 2
Correlations between androgen measurements and anthropometric measurements in Samoan women.

	N	SHBG (nmol/L)	TT (nmol/L)	FAI
Age (years)	494	0.094*	−0.179	−0.174
Weight (kg)	494	−0.411	0.167	0.395
BMI (kg/m^2)	494	−0.418	0.200	0.420
Abdominal circ (cm)	491	−0.382	0.171	0.377
AHR	491	−0.188	0.066 (NS)	0.175
AHtR	491	−0.374	0.188	0.381
Arm circ (cm)	492	−0.400	0.150	0.377
Hip circ (cm)	492	−0.387	0.176	0.384
Calf circ (cm)	492	−0.383	0.178	0.382

SHBG = sex hormone binding globulin, TT = total testosterone, FAI = free androgen index. AHR = abdominal circumference-to-hip ratio, AHtR = abdominal circumference-to-height ratio, BMI = body mass index, circ = circumference. All Pearson's correlations were significant at the $p < 0.01$ level, unless noted otherwise.

* $0.01 < p < 0.05$, NS = not statistically significant.

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Table 3
Androgen measurements stratified by body mass index (BMI) categories.

BMI category[a]	N	Androgen measurements relative to normal BMI category[b]			
		SHBG (nmol/L)	Total testosterone (nmol/L)	FAI	95th FAI percentile
Normal	55	81.8 (0.2) referent	1.2 (0.2) referent	1.5 (0.4) referent	6.7
Overweight	132	58.6 (0.2) [−28%][c]	1.4 (0.2) [+17%]	2.4 (0.4) [+58%]	9.5
Obese	307	38.6 (0.3)[d] [−53%]	1.7 (0.2)[e] [+42%]	4.4 (0.4)[d] [+84%]	19.0

BMI = body mass index, SHBG = sex hormone binding globulin, TT = total testosterone, FAI = free androgen index.
[a] Polynesian BMI classifications (in kg/m^2): Normal <26, Overweight 26–32, Obese >32.
[b] Results are reported as median (logarithmic standard deviation).
[c] Brackets [ ] indicate percent difference from referent value.
[d] $p < 0.001$ for all comparisons (Normal:Overweight, Normal:Obese and Overweight:Obese).
[e] $p = 0.10$, 0.0016 and 0.027 for the three comparisons (Normal:Overweight, Normal:Obese and Overweight:Obese).

specifically demonstrate the natural existence of clusters in the data, which was not described in either study. Our Samoan results indicate that this type of clustering analysis is not appropriate for our data given the highly normal distribution of the histogram of log-transformed FAI values (Fig. 1) with no naturally existing clusters.

With the paucity of literature defining normative androgen data among populations, we are limited to the two aforementioned studies among Chinese and Iranian populations for comparison of our findings. Using k-means clustering, the Chinese study establishes a cut-off of FAI > 6.1 among n = 1526 Chinese women aged 20–45 years. However, they also report the 95th FAI percentile, which is 6.4 among a healthy subset of n = 944 Chinese women with regular menstruation, normal metabolic hormones, absence of polycystic ovaries, and other criteria [12]. Using the cut-off of FAI > 6.1 to define HA, the prevalence of HA among the total Chinese study sample is slightly above 10%. In the study among Iranian women of reproductive age, the FAI by k-means clustering is 8.76, which corresponds to a HA prevalence of 5% [26]. Thus, despite a similar cut-off to the Iranian population and a higher cut-off to the Chinese population, our FAI > 8.5 cut-off yields a relatively higher HA prevalence of 14% among Samoan women. A major contributing factor to the population differences in HA prevalence may be the substantially higher average BMI level among Samoans (34.2 kg/m^2) compared to the average BMI values in the Chinese (20.8 kg/m^2) and Iranian (26.8 kg/m^2) studies [12,26], although neither of these studies reported the mean BMI for the healthy group in which they defined HA.

Our HA prevalence of 14% contrasts with our earlier report of women studied in 2002–03 from both Samoa and American Samoa in which a prevalence of 67% for HA was reported based on a median FAI cut-off of 4.3 among women who had normal menstrual cycles with normal BMI based on Polynesian BMI classifications, BMI < 26 kg/m^2 [27]. This study improves on a weakness in the earlier study by using a 95th FAI percentile cut-off instead of a median cut-off, which likely overestimates the HA prevalence. We also have a larger reference group by using the lowest BMI tertile, instead of normal BMI based on Polynesian criteria. As shown in Table 3, using the 95th percentile among women with normal BMI (n = 55) would result in a cut-off of FAI = 6.7. Despite using a reference group with higher mean BMI for our definition of normal FAI, it is important to note we get a relatively high hyperandrogenemia prevalence (14%) compared to the studies in the literature aforementioned.

We further expand on our earlier study by comparing women who have biochemical HA with women who have normal androgen levels,

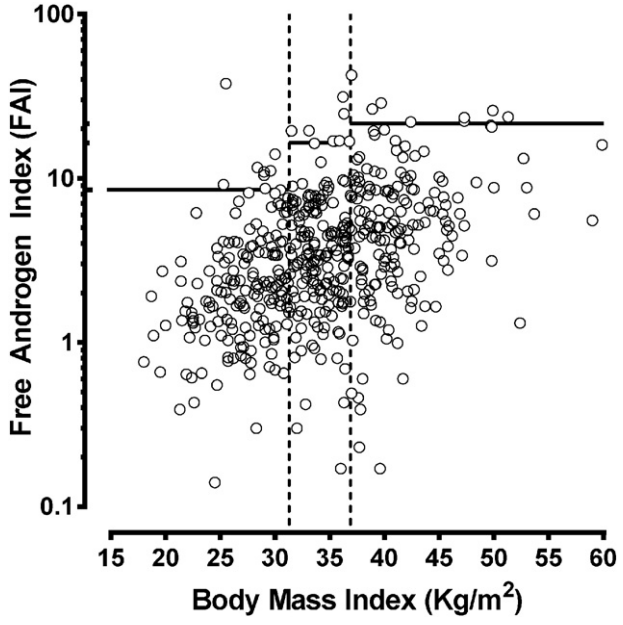

Fig. 2. Scatterplot of body mass index (BMI) versus free androgen index (FAI). The BMI (x-axis) is divided into tertiles shown by the vertical dashed lines (cut-off levels of 31.3 and 36.9) with mean BMI levels in the three groups of 26.9, 34.0 and 41.7 kg/m^2 (n = 165, 165 and 164 observations, respectively). The 95th percentiles of FAI in these three groups are shown by horizontal solid lines at 8.5, 16.4 and 21.5, respectively.

Table 4
Age, anthropometry, and metabolic hormones by hyperandrogenemia (HA) status.

	Normal androgen mean (SD)	N	Hyperandrogenemic mean (SD)	N
Age (years)	32.6 (4.5)	425	30.5 (4.4)[a]	69
25.0–29.9 (%)	32.9		56.5[a]	
30.0–34.9 (%)	32.9		23.2	
35.0–39.9 (%)	34.1		20.3	
BMI (kg/m^2)	33.4 (6.4)	425	39.2 (6.9)[a]	69
Normal (%)	12.5		2.9[a]	
Overweight (%)	29.2		11.6	
Obese (%)	58.4		85.5	
Abd circ (cm)	103.4 (14.0)	422	114.0 (13.4)[a]	69
Obese abd circ (%)	84.4	422	100[a]	69
AHR	0.91 (0.06)	422	0.93 (0.06)[b]	69
Obese AHR (%)	84.8	422	89.9	69
AHtR	0.64 (0.08)	422	0.70 (0.08)[a]	69
Obese AHtR (%)	92.9	422	100.0[a]	69
Weight (kg)	88.4 (18.5)	425	103.4 (20.4)[a]	69
Arm circ (cm)	36.5 (5.4)	423	40.3 (5.3)[a]	68
Hip circ (cm)	113.1 (11.7)	423	122.4 (12.0)[a]	69
Calf circ (cm)	42.4 (4.5)	423	46.1 (4.6)[a]	69
Cholesterol (nmol)[c]	4.68 (0.07)	401	4.79 (0.08)	64
Triglycerides (mg/dL)[c]	0.93 (0.21)	401	1.13 (0.16)[a]	64
Adiponectin (μg/mL)[c]	5.0 (0.2)	401	4.0 (0.2)[a]	64
Glucose (mmol)[c,d]	4.7 (0.1)	385	4.6 (0.1)	59
Insulin (μU/mL)[c,d]	12.2 (0.3)	385	19.5 (0.3)[a]	59
HOMA-IR[c,d]	2.4 (0.3)	385	3.8 (0.3)[a]	59
Type 2 diabetes (%)	4.0	401	7.8	64

BMI = body mass index, Abd = abdominal, Circ = circumference, AHR = abdominal circumference-to-hip ratio, AHtR = abdominal circumference-to-height ratio. Based on WHO criteria [16], Obese Abd: Abd circ > 88 cm, Obese AHR: AHR > 0.85, Obese AHtR: AHtR > 0.5. HOMA-IR = homeostatic model assessment-estimated insulin resistance.
[a] $p < 0.01$.
[b] $0.01 < p < 0.05$.
[c] Reported as median (log SD).
[d] Excluding diabetic women (n = 21).

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finding significantly larger mean anthropometric measures of both central and peripheral obesity as well as higher median values of cardiometabolic risk factors among women with HA. Thus, our definition of hyperandrogenemia is capturing a biological difference, although it should be noted that this HA group may be representing a more severe form of the condition, based on the higher FAI cut-off resulting from our reference group. Due to limited existing data in Samoa and the lack of a standardized approach, we use this statistically and biologically conservative method in order to proceed in understanding metabolic correlates of hyperandrogenemia among Samoan women.

The present study also improves on our earlier work by using self-reported data on contraceptive use that was not available in the previous 2002–03 sample. This data allowed us to exclude women who may have been using contraceptive injections. Contraceptive injections can result in irregular menstruation and altered androgen levels [12,14]. We are thus able to better understand the relationship between adiposity and androgens and accordingly establish normative androgen data, quantitatively taking into consideration BMI.

There are very few publications that address the definitions of normal and high serum androgen levels among women in the context of adiposity. The present methodology provides a rationale that can be applied to obtain population-specific definitions of hyperandrogenemia in other ethnicities. Because adiposity levels are increasing in women, understanding the associations among HA and related conditions such as menstrual irregularity, polycystic ovarian syndrome (PCOS), and cardiometabolic risk factors can be improved by accounting for BMI when considering the influence of HA. In addition, a tentative diagnosis of HA using such FAI criteria may be useful, especially in populations where clinical examination for HA is not routinely performed, such as in Samoa. Further studies will examine the reproductive condition of Samoan women using this new and carefully defined FAI cut-off.

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