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Meta-Analysis

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Nonalcoholic Fatty Liver Disease in Women and Girls With Polycystic Ovary Syndrome

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Abbreviations: BMI, body mass index; FAI, free androgen index; HOMA-IR, Homeostatic Measure of Insulin Resistance; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PCOS, polycystic ovary syndrome.

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Abstract

Context: Nonalcoholic fatty liver disease (NAFLD) describes a spectrum of liver damage due to excessive hepatic lipid accumulation. Recent research has demonstrated a high prevalence of NAFLD in women with polycystic ovary syndrome (PCOS).

Results: Strong associations independent of body mass index (BMI) have been found between high androgen levels characteristic of PCOS, as well as insulin resistance, and the presence of NAFLD in these women, suggesting that these factors contribute to liver injury more significantly than obesity. Current studies indicate the occurrence of NAFLD in normal weight women with PCOS in addition to the commonly researched women who are overweight and obese. While the majority of studies address NAFLD in adult, premenopausal women (ages 25-40 years), the occurrence of NAFLD in young and adolescent women has gone largely unaddressed. Research in this field lacks diversity; a majority of studies either focus on populations of White women or are missing demographic information entirely.

Conclusions: Future studies should include larger, more racially and ethnically inclusive populations and particular attention should be paid to how excess androgens and insulin resistance contribute to the increased risk of NAFLD seen in women with PCOS of varying weights, ages, and ethnicities.

Objective and Methods: Here, we review NAFLD in women with PCOS with subsections focused on the impact of hyperandrogenism, BMI, insulin resistance and age. Most notably, we present the most up-to-date racially and ethnically diverse worldwide prevalence of NAFLD in women with PCOS compared with women without PCOS (51.56% vs 29.64%, $P < .001$, respectively).

Key Words: Polycystic ovary syndrome, metabolic syndrome, adolescent, race, ethnicity

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Nonalcoholic Fatty Liver Disease

Nonalcoholic fatty liver disease (NAFLD) represents a spectrum of progressive liver damage, ranging from mild fatty liver to severe fibrosis and ultimately cirrhosis (1). NAFLD is diagnosed by fatty liver content greater than 5% (1) and confirmed by histology or imaging (2). In the initial stages, irregularities in lipid metabolism lead to fat accumulation in the liver, which increases the risk for further injury and inflammation (3). Over time, the fat accumulation can cause liver cell injury and death as it progresses to nonalcoholic steatohepatitis (NASH) (3); this stage is evidenced by histological ballooning (1). Further inflammation can lead to severe fibrosis and eventually cirrhosis of the liver (2).

Nonalcoholic fatty liver, the earliest stage of NAFLD, is often nonthreatening and can be reversible (1, 2). Comparatively, more progressed stages of NAFLD have been shown to develop into NASH almost twice as quickly with 20% of these patients progressing fully to cirrhosis (1). With NASH-related cirrhosis being 1 of the top 3 indications for liver transplant in the United States and NAFLD patients at high risk for cardiovascular and cerebrovascular disease (1), an understanding of this disease is necessary in order to identify it at its early stages and to prevent further progression.

The prevalence of NAFLD is approximately 6% to 35% worldwide, and various risk factors for its development have been established (4). Notably, in individuals with obesity and type 2 diabetes, the rates of NAFLD increase dramatically to 75% (2). Additional risk factors include dyslipidemia, increasing age, ethnicity, and, most significant to this review, polycystic ovarian syndrome (PCOS) (4).

Polycystic Ovarian Syndrome

PCOS is the leading cause of infertility in women (5) and affects 9% to 12% of women using the National Institutes of Health or Androgen Excess Society criteria and 12% to 18% of women using Rotterdam (6). Women with PCOS often present with anovulation, hirsutism, irregular or absence of menstrual periods, and alopecia (2). It has been hypothesized that the high levels of androgens produced by the ovaries lead to excessive release of insulin, although additional mechanisms are currently being investigated (2). This compensatory hyperinsulinemia resulting from IR will then feedback onto the brain and ovaries to produce more androgens, ultimately continuing the vicious cycle of PCOS (2). Whether IR, hyperandrogenism, or some other factor comes first and/or plays a bigger role in the pathophysiology of PCOS is not known. Longitudinal studies starting in younger childhood through adolescence and/or interventional studies are needed to understand this better.

Prevalence of NAFLD in PCOS

Associations between NAFLD and PCOS have been noted and confirmed by recent research (2). Current research places the prevalence of NAFLD in women with PCOS at 15% to 55%, with the wide range likely due to differences in diagnostic criteria (2). While the majority of these studies included only overweight (body mass index [BMI] = 25-30) and obese (BMI > 30) women with PCOS (2), research has also begun to show an increasing prevalence of NAFLD in normal weight (BMI < 25) women as well (2, 7-9), which draws attention to the possibility that NAFLD development may be independent of BMI. NAFLD has additionally been observed in young girls and adolescents with PCOS, suggesting that its development may occur at any age (2, 10-12). Here, we present a new ethnically diverse worldwide prevalence of NAFLD in women with PCOS.

Inclusion Criteria for Articles Reviewed

In February 2021, multiple PubMed searches were made utilizing the key words "NAFLD" and "PCOS" (in their different variations (such as hepatic steatosis and fatty liver for NAFLD), spelled out fully and/or abbreviated), along with a third key word that varied. In each search, excluded articles consisted of the following: review papers, clinical trials, repeated articles from previous searches, reports, clinical guidelines, studies not conducted in humans, and articles that were not accessible. Table 1 outlines each set of search results as well as the excluded articles.

Common Associations of NAFLD

Hyperandrogenism

Previous research has shown that high androgen levels may play a role in the increased rate of NAFLD seen in women with PCOS (8, 13-18). In a prospective study of Asian Indian women with PCOS, for example, researchers found testosterone levels to be significantly elevated in women with both PCOS and NAFLD compared with women with PCOS alone ($P < .01$), and claimed hyperandrogenism to be an independent predictor of NAFLD (14). In this study, it is noteworthy that the researchers used total serum testosterone levels as a measure of androgens. Free testosterone or the free androgen index (FAI) more accurately measures biologically available androgens and thus the association between androgens and NAFLD may not have been as strong if either of these was measured instead. Yet when indicators of free androgens were specifically measured in a study conducted by Vassilatou et al., increased FAI values were found to be significantly associated

Table 1. PubMed searches and inclusion/ exclusion information

Search number	1st key word	2nd key word	3rd key word	Number of articles	Number excluded	Number included
1	NAFLD			25 515	25 485	30
2	PCOS			18 604	18 572	32
3	NAFLD	PCOS		209	184	25
4	NAFLD	PCOS	Race	5	1	4
5	NAFLD	PCOS	Ethnicity	6	5	1
6	NAFLD	PCOS	Hyperandrogenism	34	26	8
7	NAFLD	PCOS	Androgens	45	40	5
8	NAFLD	PCOS	Adolescent	27	26	1
9	NAFLD	PCOS	Lean	7	5	2
10	NAFLD	PCOS	BMI	47	43	4
Total PubMed articles analyzed in body						32
Additional PubMed "NAFLD" + "PCOS" articles included in figures						47
Additional PubMed articles for background information						22[a]
Total articles cited						101

In February 2021, multiple PubMed searches were made utilizing the key words "NAFLD" and "PCOS" (spelled out fully and/or abbreviated), and variations with a third key word. In each search, excluded articles consisted of the following: review papers, clinical trials, repeated articles from previous searches, reports, clinical guidelines, studies not conducted in humans, and articles that were not accessible. There were 47 additional studies cited in the figures only to analyze racial ethnic inclusion.

[a]Background articles were not selected with the same inclusion and exclusion criteria as the other articles. For instance, some background articles are review articles on PCOS or NAFLD; and some background articles are not focused on NAFLD or PCOS such as the articles addressing the social construct of race or the use ancestry technologies.

(P = .002) with an increased prevalence of NAFLD (19); this parallels the first study's findings and further illustrates how hyperandrogenism may contribute directly to the development of NAFLD.

More recent studies have replicated the Vassilatou et al. findings (8, 15, 20, 21). A study conducted in China observed that as the FAI quartile of Chinese women with PCOS increased, the prevalence of NAFLD increased as well (20). Another study noted a similar trend: not only was liver injury associated with high androgen levels in women with PCOS, but the highest quartile of FAI levels also represented the highest risk of liver damage as well (21). Thus, not only is there an evident association between hyperandrogenism and hepatocellular injury risk, but the risk seemingly increases as androgen levels increase as well. An additional study found associations between elevated FAI and NAFLD, ultimately concluding women with hyperandrogenic PCOS to be a specific phenotype that carries a distinct, increased risk for the development of fatty liver disease (15). Finally, an additional group of researchers demonstrated similar results to Vassilatou, as high testosterone levels were associated with an increased risk of NAFLD (8). However, these researchers used hazard ratios (HRs) to represent risk of NAFLD, which are projections based on patterns found in their large-scale retrospective chart review and do not reflect the true incidence of NAFLD. Overall, current research suggests that high levels of bioavailable androgens play a prominent role in PCOS-related NAFLD, as they were shown to be strongly associated with not only the presence of NAFLD but the risk for its development as well.

Various studies have additionally noted the association between high androgen levels and increased presence of NAFLD to be independent of other factors (14, 15, 19-22). The association between higher FAI and liver fat/NAFLD found by Cai et al. was demonstrated to be independent of obesity (20). Chen et al. noted that their marker of livery injury, alanine aminotransferase levels, remained significantly associated with hyperandrogenism following adjustment for obesity (21), while Harsha Varma et al. found hyperandrogenism, in addition to IR, to be an independent predictor of NAFLD (14). Other researchers observed that the higher liver fat present in women with hyperandrogenic PCOS than in women with normoandrogenic PCOS persisted even after adjustment for both Homeostatic Measure of Insulin Resistance (HOMA-IR) and BMI (15). Two studies additionally demonstrated that the association found between increased FAI values and the presence of NAFLD remained after adjustment for BMI and waist circumference (19) and IR (22). Together, these researchers' findings collectively show that not only is hyperandrogenism significantly associated with the presence of NAFLD in women with PCOS, but that this association is often independent of other factors such as BMI, central adiposity, and/or IR.

It is important to note that some studies have not shown this association between hyperandrogenism and NAFLD (11, 23). Researchers in 1 such study demonstrated that

while 49% of the adolescent girls with PCOS had evaluated hepatic steatosis compared with 14% in controls, the percent liver fat did not correlate with measured androgen levels (11). These researchers brought attention to their inability to measure free testosterone in all subjects and speculated that, had they been able to, they may have indeed found an association between steatosis and androgens (11). Another study noted a significant prevalence of NAFLD within a group of Chinese women with PCOS (32.9%) compared with women without PCOS (18.5%), but found that it was not associated with hyperandrogenism (23). It is unclear which form of testosterone Qu et al. measured, which could potentially change the results of this association as measurements of different forms of testosterone may lead to differences in the association between androgens and NAFLD.

Overall, numerous studies have shown strong associations between high androgen levels and the presence of NAFLD in women with PCOS. A few researchers have not found similar associations, but these discrepancies can likely be attributed to inconsistencies in the form of testosterone measured. The increasing data in support of the independent role that hyperandrogenism plays in NAFLD development necessitates further research. Gaining a deeper understanding of the role that excess androgens play in NAFLD can lay the groundwork for potential areas of clinical intervention. Future studies should focus on bioavailable androgens in diverse populations, measured via FAI and free testosterone, while particular attention should be paid to how differences in BMI contribute to NAFLD, if at all.

Body Mass Index

Despite the establishment of BMI as a risk factor for development of fatty liver, current literature has revealed NAFLD to be present among normal weight (BMI < 25) women with PCOS (7-9, 22). One group of researchers found that 39% of the women with PCOS who had hepatic steatosis were lean and also noted NAFLD to be common in women with PCOS independent of BMI (7). Another study looked at a group of nonobese women and found that 5.5% of women with PCOS had NAFLD compared with 2.8% in women without PCOS ($P = .027$) (22). Although the design of this study did not allow for comparisons of NAFLD presence between nonobese and obese women, these researchers highlight the important fact that NAFLD was indeed present in the group of nonobese women. Furthermore, a longitudinal study of over 180 000 women in the UK found the HR for NAFLD development in lean women with PCOS to be nearly 2-fold higher than that of BMI-matched women without PCOS (HR = 1.92, $P < .031$). Although this study utilized HRs, which are projected risks and not true incidence of NAFLD, it still highlights how normal weight women with PCOS are at risk for NAFLD and indicates that a mechanism other than weight or BMI contributes to its development (8). Finally, a study evaluating a group of Mexican women with PCOS additionally found PCOS to be a risk factor for development of NAFLD regardless of BMI (9). Lean women with PCOS had a 14-fold higher presence of NAFLD compared with lean women without PCOS (60.9% vs 4.3%), while obese women with PCOS had only a 1.25-fold increase in NAFLD presence compared with their BMI-matched controls (76.9% vs 61.5%) (9). Interestingly, when the researchers combined all BMI categories and reported the presence of NAFLD in all PCOS women compared with all non-PCOS women, there was only a 2-fold increase (69.3% vs 34.6%) in NAFLD. Differing greatly from the 14-fold and 1.25-fold increases seen when separated into lean and obese categories, respectively, this reported 2-fold increase in NAFLD thus suggesting the need for additional considerations when interpreting BMI-matched data in these types of clinical studies. It is also important to note that lean women with PCOS (60.9%) had the same prevalence of NAFLD as obese women without PCOS (61.5%). This study used Rotterdam criteria to diagnose PCOS and thus the PCOS group may contain nonhyperandrogenic women. Additionally, the obese group without PCOS may include hyperandrogenic women. If this study were instead grouped by hyperandrogenemia instead of PCOS, the lean PCOS group may have had a higher incidence of NAFLD and the obese non-PCOS group may have had a lower presence of NAFLD. Examining the data in this manner is a potential area of future study. Nonetheless, these findings clearly show that NAFLD may have 2 independent mechanisms of development in females, 1 that is obesity related and 1 that is obesity independent and PCOS related as adding one to the other only minimally increased NAFLD. The prevalence of NAFLD in lean women with PCOS suggests that there is likely another mechanism at play, independent of obesity. Indeed, 1 study using a lean mouse model of PCOS proposed this obesity-independent mechanism to be associated with the androgen receptor's disruption of hepatic insulin signaling (24). Future research is needed to develop and expand on these data.

Certain studies have found no association between obesity and the presence of NAFLD in women with PCOS (14, 18, 21). Chen et al. noted that the increased risk of liver injury that they discovered in women with hyperandrogenic PCOS was independent of obesity (21),while Harsha Varma et al. observed that between PCOS women with and without NAFLD, anthropometric markers of obesity were not significantly different. The latter study concluded

that obesity was not an independent predictor of NAFLD, which they noted to be in accordance with other recent studies (14). It is important to note that Harsha Varma et al. and Chen et al. studied a group of Asian Indian and Taiwanese women with PCOS, respectively, and due to metabolic differences within different races and ethnicities (25), the findings of these studies should be limited to the ethnicity of each respective cohort. Comparatively, a meta-analysis of studies from 12 different countries (although none from Africa) noted that women with PCOS were twice as likely to develop NAFLD and that this risk was independent of obesity (18). Overall, these studies bring attention to the point that in a substantial number of women with PCOS, BMI was shown to contribute insignificantly to their development of NAFLD.

Even though much of the recent research has shown that the risk for NAFLD development in women with PCOS is likely independent of BMI, certain studies have found an association between the 2 (23, 26-28). One study conducted in China found that the prevalence of NAFLD was significantly higher in obese women (63.51%) than in nonobese women with PCOS (15.79%) (28), and another study found significantly higher NAFLD markers in overweight/obese PCOS women, but not in lean PCOS women, when compared with BMI-matched controls (26). However, researchers of the former study defined obese as BMI > 25, which is a lower threshold than other reviewed studies and thus more women with NAFLD may be included in the obese category than nonobese (26). Finally, a study conducted in India demonstrated that BMI was associated with the presence of NAFLD in women with PCOS (27), while a study in China found that the prevalence of mild and moderate NAFLD in women with PCOS increased significantly with BMI (23). Together, these studies suggest that BMI alone is a significant risk factor for developing NAFLD and that normal weight women may not need to be screened. Yet, Karoli et al. (27) evaluated overweight/obese women only and did not include any with normal weight BMI. In addition, Qu et al. (23) drew participants who were all ethnically Han Chinese and lived in the same middle eastern region of China (Shandong Province); although this allows for a uniquely homogenous study population, the lack of diversity also renders the findings less extendable to other races and ethnicities.

Recent research has demonstrated a significant presence of NAFLD in women with PCOS and noted this association to be independent of BMI and obesity. A few studies offer data that challenge this theory, but methodological inconsistencies and study population demographics weaken the interpretation of their findings. Due to the documented presence of NAFLD in normal weight women with PCOS, clinicians should extend screening practices to include these women of lower BMI, and future research should focus on diverse populations of normal weight women with PCOS to build on the present findings.

Insulin Resistance

Current literature additionally focuses on IR commonly found in women with PCOS and the extent to which this condition may influence the development of fatty liver. The current paradigm states that excess lipids lead to insulin resistance which results in metabolic complications (NAFLD, cardiovascular disease, type 2 diabetes) (29). Multiple studies have found associations between IR and fatty liver in women with PCOS (7, 19, 23, 27, 30-32). Gambarin-Gelwan et al. noted that women with IR had a higher prevalence of NAFLD than women without IR (7). In another study, researchers found significantly higher measures of IR in women with PCOS and hepatic steatosis than women with PCOS alone (HOMA-IR = 4.66 ± 2.78 and 2.26 ± 1.45, respectively, $P < .001$) (19). Three additional studies add further support to this, as women with PCOS and hepatic steatosis were found to have significantly higher levels of IR than women with PCOS alone in each study (23, 27, 31).

Several studies have shown that not only is IR associated with NAFLD presence in women with PCOS, but that it contributes to fatty liver in an independent manner as well (12, 14, 28, 30). Targher et al. found significantly decreased insulin sensitivity in women with both PCOS and suspected NAFLD (measured via alanine aminotransferase levels) compared with women with PCOS alone (12). The study conducted by Harsha Varma et al. additionally showed IR and hyperandrogenemia to be the only 2 independent predictors of NAFLD in a cohort of women with PCOS. Interestingly, IR was the stronger predictor of the 2 (14). Finally, 2 additional studies found IR (evidenced via HOMA-IR) to be independently associated with the presence of NAFLD in women with PCOS as well (28, 30). The latter of these studies also noted that serum androgen levels were not associated with NAFLD (30), which differs from Harsha Varma et al.'s findings. This discrepancy illustrates the complexity of these interactions and the need for more focused research in order to elucidate the cause-and-effect relationship between hyperandrogenism, IR, and NAFLD.

Various studies have yielded results strong enough to suggest that IR is a major contributing factor to NAFLD (17, 23). Petta et al. found an independent association between insulin sensitivity index and steatosis in both obese and nonobese PCOS patients and consequently concluded that IR is a key player in liver damage in PCOS (17). Another study found similar results in a cohort of Chinese women: patients with both PCOS and NAFLD

had significantly higher IR than those without NAFLD (HOMA-IR = 4.38 ± 1.73 vs 2.22 ± 1.19, respectively, $P < .001$), which led the researchers to conclude that IR is an important factor in the development of NAFLD (23).

Despite all of this support, several studies contrastingly suggest that IR may not play as significant of a role in the development of fatty liver in women and girls with PCOS as other factors (11, 15, 20, 21). Cree-Green et al. noted that while the hepatic steatosis present in their cohort of girls with PCOS correlated with n7 fatty acids and visceral fat, it did not relate to insulin sensitivity (11). In another study, researchers suggested that hyperandrogenic PCOS is a risk factor for NAFLD development independent of IR (15). Finally, 2 additional studies both demonstrated that the prevalence of NAFLD in PCOS patients increased with bioavailable androgens in a manner that was independent of IR (20, 21).

Overall, the role that IR plays in the development of PCOS-related NAFLD remains to be fully elucidated. Most of the current data support the idea that IR is a key player in NAFLD development in PCOS, but its relationship in combination with hyperandrogenism necessitates further research. Future studies should employ animal models of hyperandrogenism and IR, as well as prospective methodologies with various interventions, to study the consequent effects of these conditions on fatty liver development.

Age

Of increasing importance is the high prevalence of NAFLD among women and girls of various ages with PCOS compared to those without PCOS. From the subset of literature we examined on PCOS-related NAFLD (Fig. 1A), most studies focused on premenopausal women ages 25-40 with limited data on girls and adolescents. Figure 1 depicts the age ranges and average ages of participants from studies included in this review where data was available. Interestingly, despite the emphasis of research on adult women with PCOS, some studies have shown NAFLD to be present in girls with PCOS as well (10-12, 33). In a study of 39 obese, adolescent females with PCOS, researchers observed elevated aminotransferase levels indicative of liver dysfunction in 15.4% of the girls (10). Another study found similar results in a group of young girls with obesity, where 49% of the girls with PCOS had hepatic steatosis on imaging compared to 14% of girls without PCOS (11). It should be noted that all the girls included in these 2 studies were obese and results may have been different had girls with a wider range of BMI been included. Conversely, another study had a group of 64 women aged 16-35 in which only 8 women had a BMI greater than 26. The researchers found that the ages of the women with both PCOS and NAFLD (24 ± 4 years) were not statistically different from those of the women with PCOS alone (23 ± 5 years) (12). They further noted that the ages of both of these groups did not differ significantly from the controls, healthy women without PCOS, either. Thus as neither PCOS nor NAFLD presence was found to be concentrated in any specific age group more heavily than another, the researchers concluded that the higher prevalence of NAFLD they found in women with PCOS relative to women without PCOS was independent of age (12).

Interestingly, not all studies of young girls with PCOS observed a presence of NAFLD. In a group of women (ages 20-33 years) with PCOS, for example, researchers found no evidence of liver injury by imaging or biochemical markers in a single participant. This study included only 17 women and lacked the demographic information of any of the participants, which hinders the interpretation and thus may not represent a larger population of women with PCOS (34).

Similarly, another study noted that in their cohort of 30 girls with PCOS, only 2 (6.7%) individuals displayed fatty liver. The researchers additionally found that the presence of NAFLD was associated with age, even in the adolescent age range, as girls presenting with fatty liver tended to be older (18.0 ± 2.0 years vs 15.9 ± 0.3 years; $P = .08$), although not statistically significant (32). Because all of the girls in their study had PCOS, the researchers had no control group and thus no evaluation of the statistical significance of these data can be made to a baseline group. Furthermore, while 33% of their participants were either African American or biracial, two-thirds were White. The lack of unequal representation and the absence of any other ethnic groups limits the findings and thus conclusions should not be extended to every ethnicity.

Overall, research has revealed that in young and adolescent girls with PCOS, NAFLD is present. Because NAFLD is often reversible in its early stages, a timely detection of its presence in young girls is vital to prevent further hepatic injury and holds potential to greatly improve quality of life. Due to the very limited number of studies published in this age range, more research is needed with a particular focus on racial and ethnic participant inclusion.

Racial and Ethnic Considerations

Diversity in Research

Several studies have looked at racial and ethnic differences in metabolic syndrome in women (35, 36) and girls (33) with PCOS, but studies exploring racial and ethnic differences in women with PCOS and NAFLD are lacking. One group of researchers demonstrated NAFLD incidence of

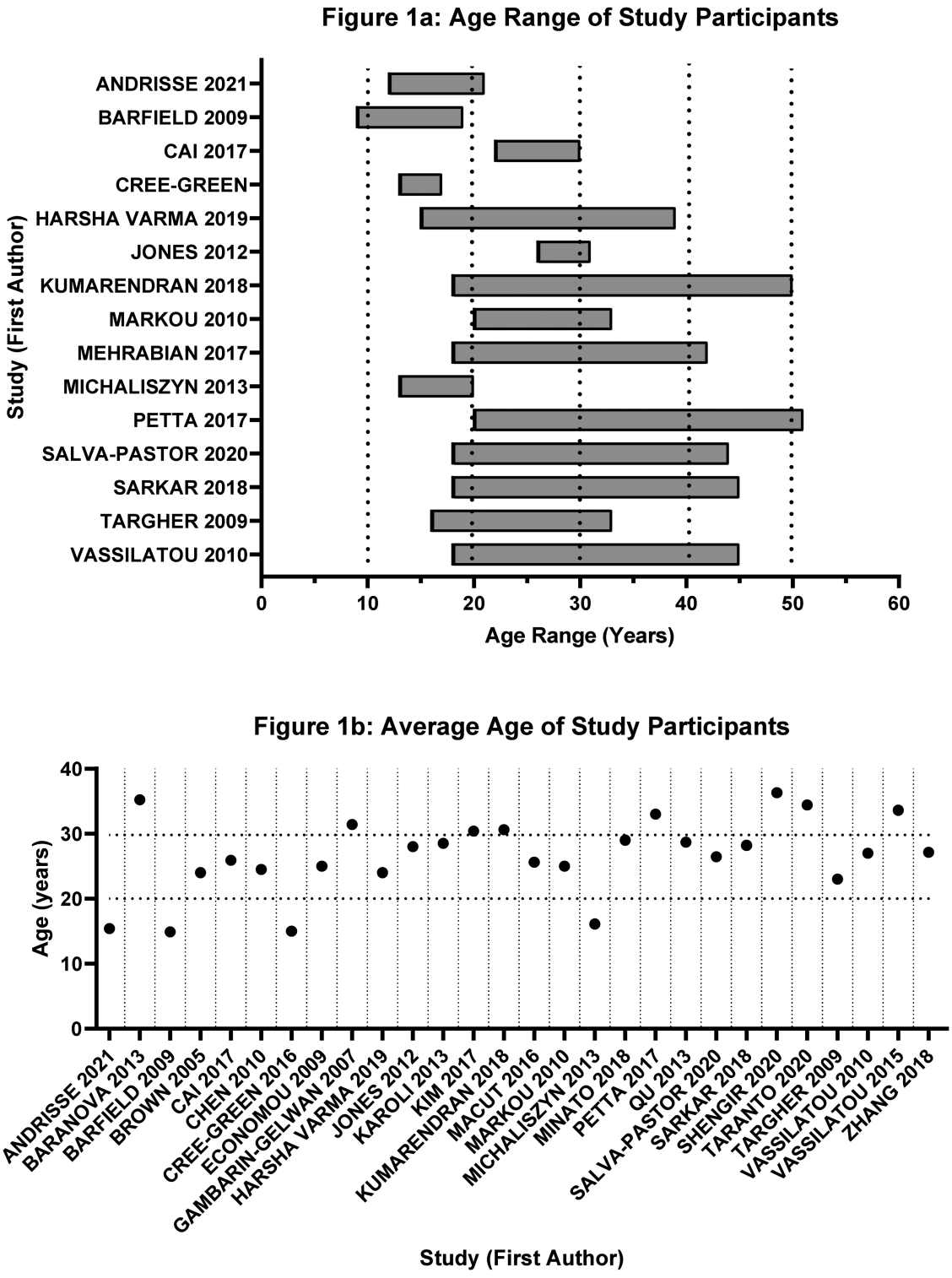


Figure 1. Details of participant ages for the studies included in this review, where provided. (A) Age range of participants. (B) Average age of participants. Of note, only 15 and 28 of the 28 relevant studies analyzed in this review provided age range and/or average age information, respectively.

50.6% in women with PCOS compared with 34.0% in non-PCOS controls in their study and subsequently encouraged clinicians to increase screening for NAFLD in all patients with PCOS (30). Due to racial and ethnic differences in metabolism (25) and the ethnically polarized composition of this study (600 Caucasian women), these researchers' findings are better directed toward White women specifically instead of all women. Similarly, while the study done by Vassilatou et al. in 2010 demonstrated for the first time the significance of bioavailable androgens

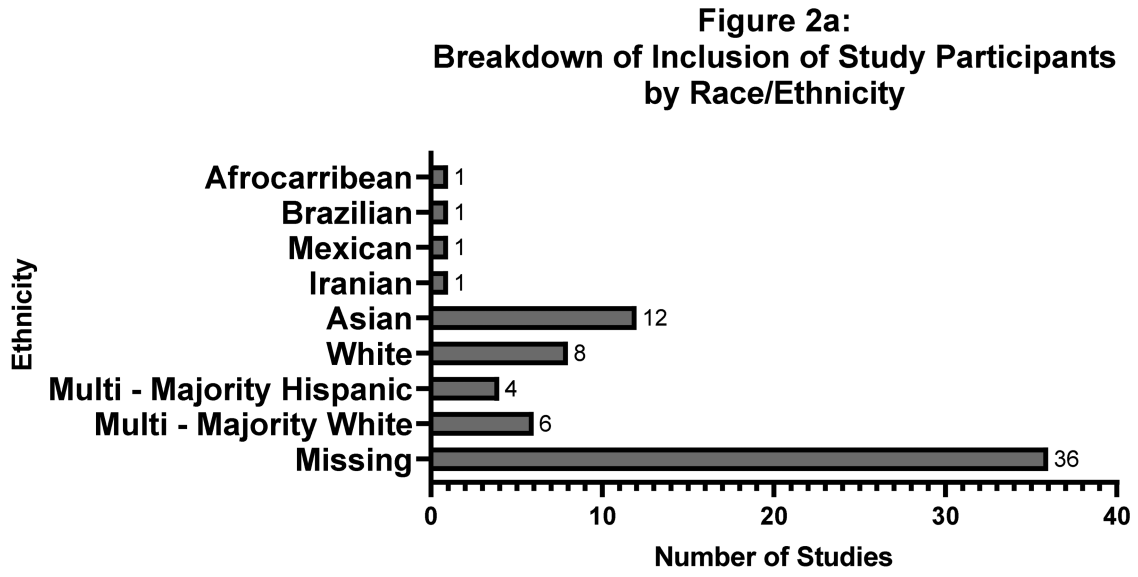
in the risk for NAFLD (19), their participant and control populations in this study and a subsequent one consisted entirely of White individuals (19, 37). These researchers recommended specific clinical practice guidelines from their data for the overall population of women with PCOS, even though their studies were specific to a single race/ethnicity. Finally, Wu et al.'s meta-analysis included studies conducted in 12 different countries spanning across the globe, yet none originated from anywhere in Africa (18). Thus, while this study does include more diversity than previous research, it exemplifies the significant lack of data in Black individuals in particular.

Reviewers and the scientific community at large need to be more vigilant towards addressing and calling out interpretative whitewashing (claiming blanket statements when only using homogenous populations that are primarily people of European decent or studies not adequately racially and ethnically diverse). Gambarin-Gelwan et al.'s study showed a presence of hepatic steatosis in lean women with PCOS (39% of the total 55% of women with NAFLD and PCOS), but comprised 38% Ashkenazi Jewish, 55% other White, 5% Hispanic, 2% Asian, and 1% African American participants (7). Thus, while this is an improvement from all-White population samples, the representation of other ethnicities remains unequal and the researchers' conclusions should not be applied to all women with PCOS. Conversely, the majority of the participants in Cree-Green et al.'s study were Hispanic (43.66%), with Caucasian following closely (38.03%), and Black (14.08%), American Indian (2.82%), and Asian (1.41%) trailing further behind (11). More diversified than previous research, this study closely matched the US Census Bureau data (https://www.census.gov/quickfacts/fact/table/US/PST045219#qf-headnote-b) for Black and American Indian populations, where the US population breakdown was Hispanic (18.5%), non-Hispanic White (60.1%), Black (13.4%), American Indian (1.3%), and Asian (5.9%). Another study included a similar demographic breakdown in adolescent girls (12-21 years) with PCOS (49.5% Hispanic, 38.6% White, and 11.9% Black) and ultimately found similar trends to the racial and ethnic differences in adult metabolic dysfunction (33).

Matching US Census Bureau data is regularly used to determine under- or overrepresentation in a field, such as the commonly known underrepresentation of Black people in academic medicine (38) (5% compared with the US Census Bureau's ~14%). However, it is estimated that non-Hispanic White people will no longer be the majority by 2042 (39, 40), thus using projected US Census Bureau data as opposed to current data could be more useful. Furthermore, the methodology of matching US Census data, or local demographic data (as in Andrisse et al.'s study (33)), to determine statistically significant scientific differences between the groups may not be the best practice. Typically, the best methodology to make statistically significant differences would be to have equal numbers in all groups (41, 42).

Whereas some studies utilize all-White or majority-White populations to make claims regarding women with PCOS everywhere, others lack demographic information entirely. When Markou et al. found no evidence of fatty liver in any of the 17 young girls with PCOS they studied in Athens, Greece, they subsequently claimed that clinicians may not need to screen adolescents for NAFLD (34). However, no demographic information was provided for any of these girls. In addition, Kumarendran et al.'s longitudinal study obtained patient information for over 180 000 individuals from a general practice electronic database in the UK (THIN Database), and yet no demographic information was provided for a single patient (8). Even when the participant populations are much smaller, as with Jones et al.'s study of 29 women in the UK, demographic information often remains missing (15). While the last 2 of these studies provided novel and insightful support for the independent role that hyperandrogenism plays in the risk for NAFLD, all 3 fall short in racial, ethnic inclusiveness. Some studies state the country or location of the study and expect the reader to extrapolate demographic data from this information. This is not good practice. Findings in 1 ethnic group may not be present in all women and thus when researchers do not have the means to include a diverse representation, they must disclose demographic information so that readers and the scientific community may take that into consideration themselves. Fig. 2 illustrates the racial/ethnic diversity of study populations from all original PCOS-related NAFLD studies in humans found from a February 2021 PubMed search (n = 70).

Notably, skin color and hair texture are adaptive traits that have been shown to have no objective criteria for defining races in humans (43). It has long been thought that using White people as a comparison group was stereotypically inappropriate and scientifically inaccurate (44). According to geneticists and anthropologists, race is a social construct (45, 46), thereby illustrating a need to possibly move to using ethnicity and national origin (ancestry) instead of only using race. Considering that tools to assess ancestry are readily available (47, 48), moving in this direction is feasible. Racial disparities exist in healthcare because of many factors including socioeconomic and cultural influences (49). Because racial groups are readily used in society it remains valuable to use these descriptions but using genetic ancestry is a more accurate scientific tool.



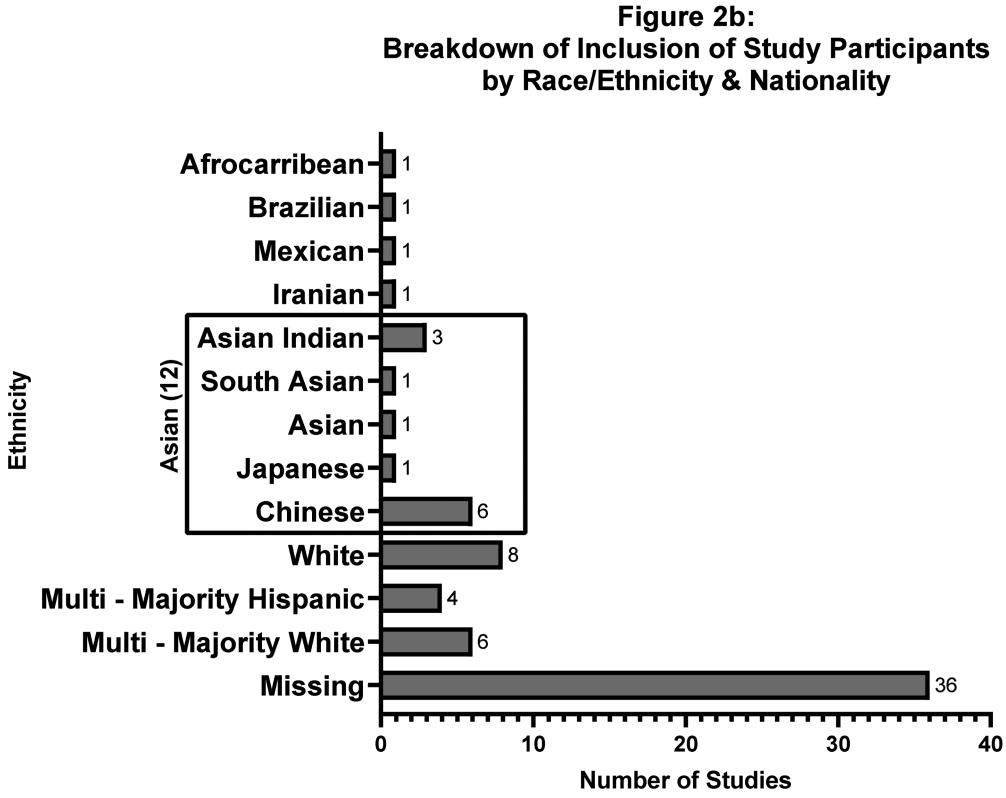


Figure 2. Demographic breakdown of study participants from a broad PubMed search of "hepatic steatosis" and "PCOS." In the initial results, 209 articles were listed. After excluding reviews, irrelevant articles (clinical guidelines, surveys, studies not done in humans), and articles that were inaccessible, 70 original studies were included and evaluated for demographic information stated within the article or tables/figures only. Multi = studies with participants from more than 1 race or ethnicity (defined here as heterogenous participant studies). (A) Demographic breakdown of study participants from included studies, with 1 broad category for Asian which includes Chinese, Japanese, Asian Indian, South Asian, and Asian as reported by researchers. (B) Demographic breakdown of study participants from included studies, categorized by specific nationalities stated within each study. Heterogenous studies included Multi: majority White (7, 32, 50, 55-57); Multi: majority Hispanic (11, 33, 58, 59). Homogenous studies, defined as a single race or ethnicity, included: Asian (excluding -Indian) (Fig 2a) (14, 16, 20, 22, 23, 27, 51, 60-64); Asian (Fig. 2B) (22); Japanese (16); Chinese (20, 23, 61-64); White (17, 19, 30, 37, 65-68); Mexican (9); Asian Indian (14, 27, 60); Iranian (31); Brazilian (69); Afro-Caribbean (70); South Asian (51); no demographic information: 36 studies were missing demographic information (8, 12, 13, 15, 26, 28, 34, 71-99). Of note, 26 of the 36 studies (72.2%) with missing demographic information were done in predominantly White countries.

Prevalence of NAFLD in Non-White Populations

Despite the emphasis of research on White populations, several studies have found a high prevalence of NAFLD in women with PCOS compared with those without PCOS across various geographic regions, ethnicities, and races (Fig. 3) (9, 20, 23, 27, 28, 31, 50-52). One study conducted in a population of Asian Indian women showed NAFLD to be present in 67% of women with PCOS compared with 25% in women without PCOS (27). In a group of Iranian women, researchers noted NAFLD to be present in 38.7% of women with PCOS vs 18.7% of women without PCOS (31). In addition, 2 independent studies conducted in China both found the rate of NAFLD to be approximately 1.7 to 1.8 times higher in women with PCOS than in women without PCOS (32.9% vs 18.5% and 44.6% vs 24.6% respectively) (23, 28). A third study in Chinese women found a slightly lower rate (1.48-fold higher) of NAFLD in PCOS women (56.23%) than non-PCOS (38%) (20). Furthermore, a group of researchers found the rate of NAFLD to be twice as high in a group of Mexican women with PCOS (69.3%) vs Mexican women without PCOS (34.6%) and established Mexican ethnicity as an independent risk factor for NAFLD development (9). Indeed, the prevalence of NAFLD (69.3%) in Mexican women with PCOS was one of the highest of all racial ethnic groups reviewed in this manuscript. Another study found that NAFLD was more than 3-fold higher in Hispanics than in non-Hispanics, and showed Hispanic ethnicity to be associated with a 2-fold higher chance of developing NASH than non-Hispanics (50). In addition, researchers in Thailand noted NAFLD to be of high incidence (39.6%) in a study of South Asian women with PCOS, thereby concluding it to be a common comorbidity in this population (51). Finally, a study that recruited from an endocrinology clinic in Brazil observed a significantly higher presence of NAFLD in women with PCOS than in non-PCOS controls (77% vs 52.5%, $P = .005$) (52). Brazilians had the highest prevalence of NAFLD in women with PCOS analyzed in this review (Fig. 3A).

Even though 1 or 2 studies cannot be used to generalize the entire population of similar ethnicity, these studies bring attention to the fact that in non-White women with PCOS across the globe, NAFLD is clearly present. These findings raise the following question: if, as research and this review have shown, there is a significant presentation of NAFLD in women of varying races and ethnicities, then why is a majority of research being conducted in primarily White women? To better illustrate this discrepancy, Fig. 3 portrays the prevalence of NAFLD found within different ethnic and racial groups of women with PCOS. Compared with Fig. 2, this disparity (NAFLD being present in a wide range of racial/ethnic groups yet primarily only being studied in White women) is clear.

Conclusion

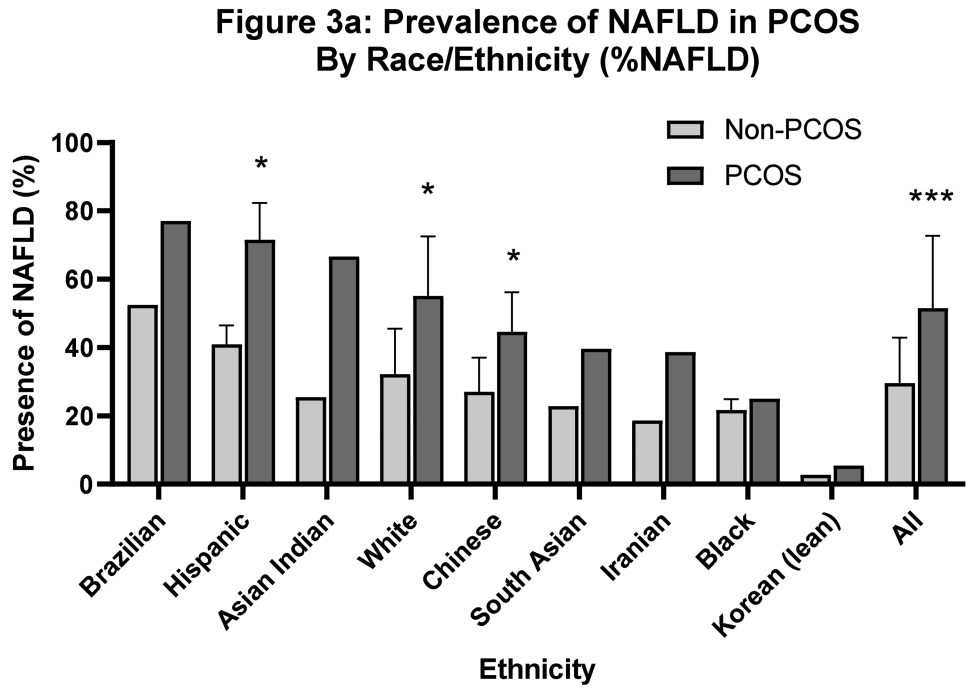
Clinical Implications

A collaboration between the American Association of Clinical Endocrinologists and the Androgen Excess Society published a summary of best practices for physicians in 2014 that highlighted the need for all women with PCOS to be screened and evaluated for metabolic syndrome (53). Despite these recommendations, a systematic review of clinical practice guidelines conducted in 2021 found several inconsistencies in the management of PCOS, including the frequency and screening criteria used for metabolic disease in these patients (54). Specifically, the researchers found that metabolic screening was more frequent in women with a BMI >25 and/or age >40 years. Taking into consideration the findings of this meta-analysis, clinicians need to change screening practices to reflect the incidence of NAFLD that is seen in women of all ages and BMI categories. Frequency of metabolic screening should increase in normal weight women as well as adolescent women and girls. Thus, all patients with PCOS will have an equal opportunity to prevent further metabolic dysfunction.

Summary

Current literature in the field of PCOS-related NAFLD has presented several common associations, most notably excessive bioavailable androgens (8, 14, 15, 18, 19, 21, 22) and IR (7, 11, 12, 14, 15, 17, 19-21, 23, 27, 30-32). Recent research has also noted a significantly higher prevalence of NAFLD in women and girls with PCOS than in those without PCOS of varying ages (10, 12) and BMI categories (7, 9, 22), which suggests that these factors may not play as important of a role in the development of fatty liver as previously thought. Because NAFLD is often reversible in its early stages, a timely detection of its presence in young girls and normal weight women in particular is vital to prevent further hepatic injury and holds potential to greatly improve quality of life. More research is needed to elucidate the relationship between hyperandrogenism and IR in NAFLD development so that future work can focus on treatment and intervention methods. All new studies should include consistencies across methodological practices and consideration of race and ethnicity in their interpretations.

Current research, while supportive of the independent role that hyperandrogenism and IR likely play in NAFLD development in PCOS women of all ages and BMI, lacks diversity. The participants included in many studies are



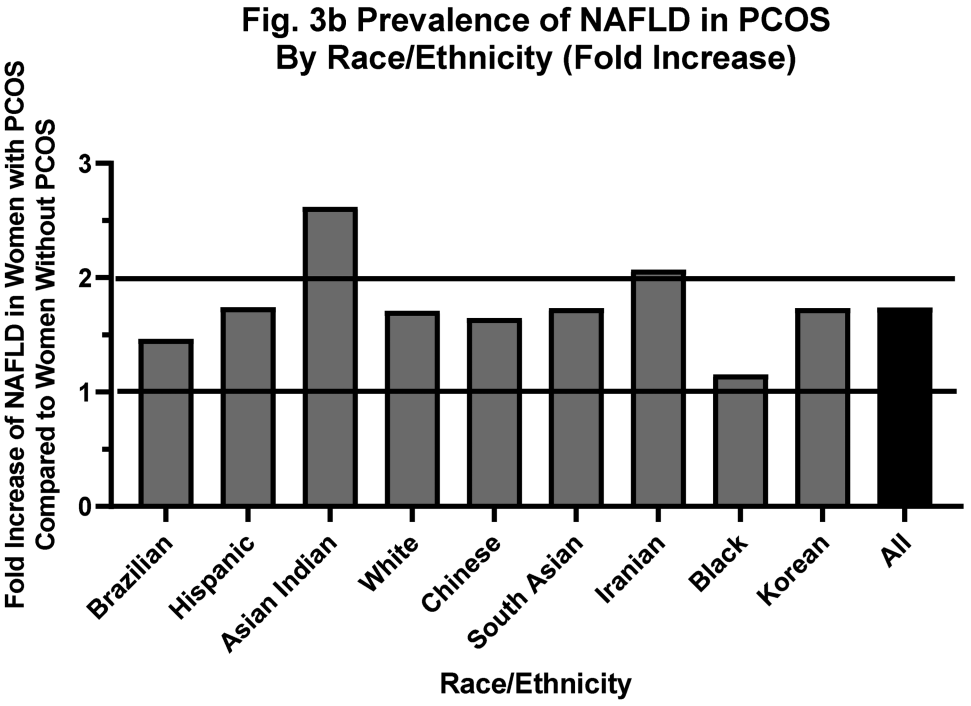


Figure 3. Prevalence of NAFLD in women with PCOS compared with women without PCOS. (A) Percentage of NAFLD incidence in women with PCOS vs women without PCOS across race and ethnicities. (B) Fold increase of NAFLD in women with PCOS relative to women without PCOS across race and ethnicities. White (17, 19, 30, 33, 37, 100, 101); Hispanic (9, 33, 50, 100, 101); Chinese (20, 23, 28); Korean (Kim et al 2017 utilized a cohort of only lean (BMI < 25) women) (22); South Asian (51, 102) (Shengir et al. did not specify which nationality of South Asian their participants were); Asian Indian (27); Iranian (31); Brazilian (52), Brazilians are considered Latinx because the country is located in Latin America, but Brazilians are not considered Hispanic because their country's primary language and ethnic nationality is Portuguese (103); Black (33, 100, 101). Two-way analysis of variance performed comparing control vs PCOS within all groups. Paired t-test performed comparing controls vs PCOS within specific groups (Hispanic, White, Chinese, All); *$P < .05$ compared with control for that group; ***$P < .001$ compared with controls for that group.

majority White, if not all White (19, 30, 37), and studies that include more diverse participant populations often lack equal and/or proportional representation and often underrepresent Black individuals in particular (7, 11, 18, 33). Finally, some studies simply do not provide demographic information for their participants at all (8, 15, 34). Due to the increased presence of NAFLD in women with PCOS in multiple races and ethnicities (9, 20, 23, 27, 28, 31, 50-52), the current practices of participant inclusion need to change. It is understandable that geographic location may pose a challenge to diverse participant recruitment; however, this challenge should be stated and addressed within the study. Dramatic diversification is warranted to not only increase representation within studies, but to account for the effect that varying ethnicities have on metabolic dysfunction.

This meta-analysis has certain limitations that are important to note. For several of the figures, statistical analysis was not possible in some groups, as only 1 study had data to analyze. While this leads to a smaller sample (n = 1) for various groups, it also highlights the dire need for further research in this field in order to build on current data. In addition, we acknowledge that some of the studies discussed in this paper likely included homogenous, non-diverse participant populations intentionally in order to study PCOS and NAFLD in women of a specific race or ethnicity.

In conclusion, NAFLD has been noted in women with PCOS of varying weights, ages, races/ ethnicities, and correlates with excess androgens and IR. Future studies should incorporate younger, leaner, and more racially and ethnically diverse populations with particular attention given to the contributions of hyperandrogenism and IR to the increased risk of NAFLD.

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