UNIVERSITY OF CALIFORNIA SAN DIEGO

Per- and polyfluoroalkyl substance (PFAS) and serum lipid levels in Ecuadorian adolescents

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of

Public Health

by

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The thesis of Michelle Guerra is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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ABSTRACT OF THE THESIS

Per- and polyfluoroalkyl substance (PFAS) and serum lipid levels in Ecuadorian adolescents

by

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Master of Public Health

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Introduction: Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals used for various purposes, including pesticide formulation. Recent evidence highlights PFAS's negative impact on human health, increasing the risk of altered serum lipids. The sex-specific relationship between PFAS and serum lipids remains unclear in adolescents. We aim to test the sex-stratified association between PFOS, PFOA, and PFNA exposure with serum lipids among adolescents.

Methods: This cross-sectional study comprised 97 adolescents 11-17 years of age from Pedro Moncayo, Ecuador. A generalized linear model was used to estimate the associations of serum PFOS, PFNA, and PFOA concentrations with triglycerides, total cholesterol, HDL, and LDL

levels. The final model was sex-stratified to assess sex-specific predictor impacts on triglycerides, with all data log-transformed.

Results: When stratified by sex, we found a significant inverse relationship between PFOS (β=-15.01 ng/dL [-24.72, -4.06]), PFNA (β=-25.49 ng/dL [-36.93, -12.00]), and PFOA (β=-16.55 ng/dL [-28.16, -3.07]),) with triglycerides in females but not in males (β=-6.12 ng/dL [-5.09, -18.65]; β=10.51 ng/dL [-1.09, 23.46]); β=0.61 ng/dL [-11.07, 13.82]), respectively). We found no sex-specific association of PFOA, PFNA, or PFOS with total cholesterol, HDL, or LDL.

Conclusion: Our findings suggest an inverse association between triglycerides and PFOS, PFOA, and PFNA among adolescent females, but not males. Our findings contribute to the sparse research on PFAS in Latin America and rural populations. Further investigation into sex-related effect modification is necessary for a comprehensive understanding of this relationship.

Introduction

Background

Dyslipidemias are a group of health conditions characterized by altered serum lipid levels, which include LDL, HDL, total cholesterol, and triglycerides. Globally, dyslipidemia affects over 50% of adults, raising significant concerns due to its association with an elevated risk of cardiovascular disease and stroke (Brown et al., 2000; Hedayatnia et al., 2020; Joshi et al., 2014; Centers for Disease Control and Prevention, 2022a; Ding et al., 2022; Khatana et al., 2020). High BMI and smoking have been identified as potential risk factors for dyslipidemia (Gebreegziabiher et al., 2021; Children's Hospital of Philadelphia, 2020; Centers for Disease Control and Prevention, 2022a). Moreover, evidence suggests exposure to per-and polyfluoroalkyl substances (PFAS) also increases the risk of dyslipidemia (Centers for Disease Control and Prevention, 2022b; Ho et al., 2022). This association may be attributed to the lipophilic properties of PFAS, facilitating interactions with lipoproteins and potentially influencing lipid metabolism (Crone et al., 2019). PFAS are synthetic chemicals used for various commercial and industrial purposes due to its hydrophobic and non-stick properties (Zuanna et al., 2021). There are over 9,000 types of PFAS that exist (National Institute of Environmental Health Sciences, 2023). Among these, perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS) have been detected in human blood samples (Centers for Disease Control and Prevention, 2022c). In addition to dyslipidemia, PFAS exposure has been linked to other adverse health effects, such as kidney disease, liver disease, insulin dysregulation, and adverse reproductive outcomes (Sunderland et al., 2019).

PFAS Exposure

The main routes of PFAS exposure in humans are through inhalation, absorption, and

ingestion (Sunderland et al., 2019). Inhalation of PFAS can occur through the indoor environment, such as dust (Hall et al., 2020). Absorption mainly comes from the use of waterproof apparel and bathing in contaminated water (ATSDR, 2022). PFAS can be ingested primarily from drinking contaminated water and consumption of seafood. Of the different routes of exposure, ingestion of contaminated water is the most prominent form of environmental exposure. This contamination occurs from sewage and waste produced by manufacturing and industrial sites (Sunderland et al., 2019). Additionally, landfill containing products with PFAS can leach into groundwater and surface water and eventually contaminate the ocean. PFAS are known persistent pollutants which can bioaccumulate in the environment (Brunn et al., 2023). On a molecular level, carbon-fluorine bonds in PFAS are exceptionally strong, making them difficult to break down (Wang & Liu, 2020). As a result, this makes PFAS difficult to eliminate, leading to bioaccumulation in the environment, especially in marine life (Zhang et al., 2019).

PFOA has been used as non-stick coatings in cookware (e.g., Teflon), water-resistant textiles, and personal care products (Kang et al., 2018; Centers for Disease Control and Prevention, 2017). These PFAS can leach out of the products and into the food or directly to the skin. Furthermore, PFOA, as well as PFOS are used in aqueous film forming concentrations and fire-fighting foams (Arias, 2015). These foams can contaminate water sources due to industrial or military site wastes. Exposure to PFNA has been linked to wax, polish, and water-proof materials (Lee et al., 2017). Similarly, these chemicals could leach out of the products leading to skin absorption and ingestion.

PFAS and Pesticides

Another potential source of PFAS exposure includes pesticide formulation (Wang et al., 2017). According to the Swedish Chemical Agency, various international studies have detected PFAS in pesticides as both an active ingredient and additive, as it functions as an effective

surfactant, dispersant, and anti-foaming agent (Swedish Chemical Agency, n.d.). Furthermore, PFAS-based pesticides, including sulfluramid, can contaminate agricultural soils (Costello and Lee, 2020). A Brazilian study on sulfuramid revealed it contained PFOS as an active ingredient (Nascimento et al., 2018). Additionally, the U.S. Environmental Protection Agency (EPA) reported the detection of PFOA and PFOS in insecticides, such as sumithrin and piperonyl butoxide (Anvil 10+10) (Bennett, 2020). Moreover, PFNA and PFOA were discovered as contaminants in approximately 30% of plastic containers used for agricultural pesticides due to fluorination, a process that reinforces plastic (Nguyen, 2022; U.S. Environmental Protection Agency, 2023). PFOS has also been detected in various insecticide formulates, including malathion, spiromesifen, and imidacloprid, which are organophosphates neonicotinoids that contaminate agricultural soils with high concentrations and persist for years in the environment. In several studies, agricultural soils that have been tested for PFAS were contaminated with PFOS, PFNA, PFOA, and other variants (Brusseau et al., 2020; Lasee et al., 2022). These findings, particularly in the agricultural setting, are a cause for concern given the health risks associated with PFAS exposure, their persistence in the environment, mobility, and bioaccumulation.

PFAS and Health

Animal Studies

Several studies with rodent models have assessed the relationship between PFAS exposure and blood lipid levels among males and females. In most animal studies, males tended to have higher concentrations of PFAS, such as PFOA and PFOS, than females. This differentiation may be due to the possibly better clearance of PFAS in females through menstruation and lactation (Jain & Ducatman, 2022). However, when stratified by sex, males and females experience different levels of health effects (Roth et al, 2021). In one study,

researchers fed mice a diet with high levels of PFOA. The results demonstrated both male and female mice experienced hypercholesterolemia and altered sterol metabolism, but females had higher concentrations of plasma cholesterol than males (Rebholz et al., 2016). In another mice-model study, mice were exposed to PFOA through diet and drinking water. The results showed PFOA exposure increased serum lipoprotein cholesterol, but the relationship was stronger in males than females (Schlezinger et al., 2020). Other animal studies that exposed mice to other PFAS resulted in hypocholesterolemia, contrary to the previously mentioned study findings (Roth et al., 2021; Butenhoff et al., 2012; Han et al., 2018; Quazi et al., 2010). Overall, various epidemiological studies have also reported sex differentiation in PFAS and blood lipids (Roth et al, 2021; Schecter et al., 2012; Olson et al., 2008). However, the mechanisms for this are not well understood (Andersen et al., 2021).

Human Studies

Several epidemiological studies have investigated the association between PFAS and lipoproteins in highly exposed adult populations. One case-control study examined this relationship in 1,945 Swedish adults who consumed drinking water contaminated with PFOS and PFOA. The findings presented higher LDL and total cholesterol levels among residents exposed to drinking water contaminated with PFOS and PFOA compared to controls who were not exposed. Overall, a direct dose-response trend was observed between serum PFAS levels and serum lipids (Li et al., 2020). Similarly, a 10-year longitudinal study of 864 Swedish participants, aged 70 years, analyzed the association between PFAS, PFOS, and PFOA, on total cholesterol, triglycerides, HDL, and LDL. The results demonstrated that triglycerides and LDL increased, while HDL decreased throughout the 10-year period (Dunder et al., 2022). In addition to studies in Sweden, other cross-sectional studies have been conducted in Italy and the US which demonstrated a direct trend between increased lipoprotein levels and PFOA, PFOS, and

PFNA (Canova et al., 2020; Batzella et al., 2022; Starling et al., 2019).

Studies Among Adolescents

During childhood and adolescence, individuals undergo rapid physical and cognitive development making them sensitive life stages and more susceptible to the adverse health impact of PFAS and other endocrine disrupting chemicals compared to adults (U.S. Environmental Protection Agency, 2023; Fenton et al., 2021). Furthermore, studies have reported that PFAS may interfere with hormonal functions of the body, such as sex hormone and lipoprotein imbalance (Lee et al., 2021).

Although there are several studies on PFAS exposure and lipoprotein levels in adults, few studies have examined this relationship among children and adolescents (Ho et al., 2022). In a 3-year surveillance study conducted in Italy, researchers evaluated the association of PFAS concentrations and cardiometabolic traits, including lipoprotein concentrations, among 6,669 adolescents and 2,693 children. The study findings presented a significant direct association between increased PFOS and PFNA concentrations with increased total cholesterol, LDL and HDL levels. Additionally, PFOA and PFHxS were significantly associated with HDL. PFOS showed a stronger dose-response effect compared to PFOA and PFHxS. However, some curves had irregular shapes (Canova et al., 2021). In Norway, a cross-sectional study assessed the relationship between PFAS and metabolic syndrome in 940 adolescents. Researchers found a direct trend between PFNA and PFOS with LDL and total cholesterol (Averina et al., 2021). In a 2022 systematic review of PFAS and lipid concentrations in the blood in both adults and adolescents, an elevated association between PFOA, PFNA, and PFOS with LDL and total cholesterol was mainly observed. While only PFOS was associated with HDL, there are inconsistencies in the literature for both adults and adolescents, such as the strength and direction of the association among PFAS variants with different lipoproteins (Ho et al., 2022).

Effect modification by age and sex

Few studies have assessed effect modification by sex and age on the PFAS-blood lipid associations among adolescents. Results are mixed among the few studies that have been conducted that test this relationship. One cross-sectional study conducted on 9,362 adolescents and children in Italy found a strong correlation between elevated PFOA, PFOS, and PFHxS with HDL among females, but not in males. Furthermore, there was a direct trend between increased PFOS and PFHxS levels with total cholesterol (TC) and LDL-C in younger females (Canova et al., 2021). Contrary to these findings, a study of 12,476 adolescents and children in the US found a statistically significant relationship between PFOA with total cholesterol and LDL in males and younger children, while there was a weaker relationship in females. Furthermore, the relationship between PFOS and HDL was statistically significant for males, but no relationship was observed in females (Frisbee et al., 2010). Further research on the role of age and gender on the PFAS-blood lipid association is warranted.

Study Objectives

The purpose of this cross-sectional study is to analyze the association between PFAS and blood lipids, in adolescents growing up in agricultural communities in northern Ecuador. We hypothesized that exposure to PFOS, PFOA, and PFNA will have a direct association with total cholesterol, HDL, LDL, and triglycerides levels among adolescents. To our knowledge, no studies have been conducted in Latin America on the adverse effects of PFAS in adolescents.

Guerra, Michelle was the primary author of this thesis. Coauthors Kayser; Georgia, Checkoway, Harvey; and Suarez-Lopez, Jose Ricardo contributed to idea formulation for all chapters and provided comments and edits on multiple drafts of this thesis.

Methods

Study Design and Recruitment

The study population was drawn from The Study of Secondary Exposures to Pesticides among Children and Adolescents (ESPINA), a prospective cohort study designed to analyze the health and developmental effects of exposures to agrochemicals on children living in Pedro Moncayo, Ecuador. Pedro Moncayo County, Pichincha province, houses a large floricultural industry. As a result, pesticides are frequently applied to crops, putting agricultural workers and nearby populations at risk of exposure (Suarez-Lopez et al., 2012).

The ESPINA cohort underwent examinations in 2008 and 2016. In 2008, participants were recruited the 2004 Survey of Access and Demand of Health Services and community recruitment from government officials in order to represent a sample of the population in Pedro Moncayo County. There were 313 boys and girls who were selected based on the following eligibility criteria: 1) 4-9 years of age and 2) exposed or unexposed to agricultural pesticides. The exposed group was defined as children who lived with an agricultural worker for a minimum of one year. The unexposed group was defined as those who either never lived with an agricultural worker, never lived in a household that stored agricultural pesticides, or never had direct contact with agricultural pesticides. In 2016, there were 545 participants. There were 300 new participants, while the remaining 245 were from the 2008 measurements and underwent follow-up examinations. The eligibility requirement included children and adolescents 11-17 years of age and were either exposed or unexposed to agricultural pesticides as defined in the 2008 criteria (Phillips et al., 2021). As part of a pilot study, we measured serum lipids in 101 participants examined in April and July-October 2016. Participants were selected into the pilot study based on having the highest and lowest measurements of acetylcholinesterase (AChE)

activity levels in 2008 to include participants with potential for having both elevated and low organophosphate pesticide exposures in 2016 (Phillips et at., 2021). Four participants were excluded due to missing PFAS measurement; therefore, the current analysis includes data of 97 participants examined in July-October 2016. **Figure 1** shows the flow chart of participants selected for the present analysis.

Data Collection

In 2008, pesticide exposure history, socioeconomic status, and demographic characteristics were collected through at-home interviews of parents, which took place in 7 schools in Pedro Moncayo County. In 2016, participants were examined in schools in April and July-October. Weight was measured using a digital scale, while height was measured in millimeters with a height board, where participants did not wear shoes or headwear. The World Health Organization (WHO) growth standards were used to calculate height-for age and BMI-for-age Z-scores (World Health Organization Multicentre Growth Reference Study Group, 2006). Participants were not asked to fast prior to their examinations. Detailed methods have been described previously (Phillips et al., 2021; Suarez-Lopez et al., 2019).

PFAS Quantification in Serum.

The biospecimens initially underwent protein precipitation. The samples were then mixed with internal standard solution and acetonitrile, then were added to microcentrifuge tubes containing 400µl of plasma. The microcentrifuge tubes were then centrifuged to extract the supernatant liquid and transfer it to 96-well plates to undergo concentration. The concentrated samples were analyzed with LC/MS/MS (Shimadzu Prominence coupled to AB Sciex 5500Q) by using electrospray ionization. Bovine calf serum was used to perform isotope dilution in order to prepare matrix-matched calibration curves and quantitation. EQM Test-mate ChE Cholinesterase Test System 400 (EQM AChE Erythrocyte Cholinesterase Assay Kit 470; EQM Research, Inc,

Cincinnati, OH) was used to measure erythrocytic AChE activity and hemoglobin concentrations (mg/dL). Blood samples were collected from a fresh finger-stick blood sample. Blood samples were instantly analyzed at ambient temperatures between 15 and 28°C, which are within the recommended range, following standard procedures (EQM Research Inc., 2003).

Serum Lipid Concentrations

Total cholesterol, HDL-Cholesterol (HDLc), LDL-Cholesterol (LDLc), and triglycerides in serum were measured using enzymatic methods (OSR6616, OSR6695, OSR6196, and OSR66118 kit; Beckman Coulter, Brea USA) using a Beckman Coulter AU680 Chemistry Analyzer at US Specialty Labs in San Diego, CA.

This study was approved by the institutional review boards at the University of California, San Diego, and Universidad San Francisco de Quito. The Ecuadorian Ministries of Health and Education also approved the study. All participants below 18 years of age provided assent to participate. Additionally, all parents of participants approved permission for their children to participate in the study and adults provided informed consent.

Statistical Analysis

The mean or percent of characteristics were calculated for all participants and stratified by tertiles of PFOS concentration. P-values for participant characteristics were computed through a generalized linear model (GLM) using a log-transformed continuous variable for PFOS concentration. The geometric mean of serum PFAS concentrations was computed both for the entire sample size and categorized by gender.

The associations between PFAS and blood lipids were calculated using generalized estimating equations (GEE). Models were adjusted for age, sex, z-score for height-for-age, z-score for BMI-for-age, AChE activity, and hemoglobin concentration. Statistical significance was determined at an alpha level of 0.05. We used log transformation on both the independent

and dependent variables since they were not normally distributed. We interpreted the coefficient as the percent increase in the dependent variable, serum lipids, for every 50% increase in the independent variable, PFAS. The percent change in lipids per 50% increase in PFAS concentration, with both independent and dependent variables log-transformed, was calculated using the following method: For each 50% increase in PFAS concentration, the serum lipid levels increase by approximately the coefficient of the regression model, multiplied by the logarithm of 1.5. This coefficient-adjusted value is derived by raising 1.5 to the power of the beta coefficient, subtracting one, and then multiplying by 100.

We tested effect modification on the association between PFAS and blood lipids by age and gender through multiplicative terms. Associations with significant gender or age interaction ($P<0.05$) were then stratified by a categorical variable of the effect modifier. SAS 9.4 was used for all analyses in this study (SAS Institute, Cary, NC).

Guerra, Michelle was the primary author of this thesis. Coauthors Kayser; Georgia, Checkoway, Harvey; and Suarez-Lopez, Jose Ricardo contributed to idea formulation for all chapters and provided comments and edits on multiple drafts of this thesis.

Results

Participant Characteristics

Table 1 presents participant characteristics by tertiles of PFOS for all participants and stratified by sex. Of the 97 participants, 54% were males and 46% were females and had a mean age of 14.7 years. The z-score height-for-age was -1.4 SD below the WHO standard. The average z-score for BMI-for-age was 0.3 SD. Males were more likely to have higher concentrations of PFOS than females and borderline direct trends were observed between PFOS concentration and AChE activity.

Table 2 illustrates the serum concentrations of PFOS, PFOA, and PFNA in the complete sample population (N=97) as well as the stratified values in males (n=52) and females (n=45). The highest concentration measured among the three PFAS was that of PFOS, with a concentration of 0.63 ng/mL among males. The lowest concentration of PFAS was 0.08 ng/mL of PFNA among females.

PFAS and Serum Lipids

There was a statistically significant inverse association between PFOA and triglycerides, with triglycerides decreasing by approximately 10.86% (95% CI: -20.14 to -0.51) for every 50% increase in PFOA (ng/mL). See **Table 4.** No significant association was observed for PFOS and PFNA with any of the serum lipid variables. However, in the associations with triglycerides, there were significant gender interactions observed with PFOS (p=0.02), PFOA (p=0.04) and PFNA (P<0.001).

The relationship between PFOS, PFOA, and PFNA with triglycerides was stratified by sex. We found a statistically significant relationship between PFOS, PFOA, and PFNA with triglycerides in females but not in males (**Table 4**). Of the three PFAS, PFNA and triglycerides

exhibited the most significant inverse trend. In females, for every 50% increase in PFOS (ng/mL), triglycerides decreased by approximately 15.01%. Similarly, for every 50% increase in PFOA or PFNA, triglycerides decreased by 16.55% and 25.49%, respectively. No evidence of curvilinear associations was observed in any of the models.

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Discussion

Our findings revealed that PFOS, PFNA, and PFOA were inversely related to triglyceride concentrations in female adolescent participants, while this association was not observed in adolescent males, in a study population in which males had higher PFAS concentrations than females. We found no associations of PFOA, PFNA, or PFOS with total cholesterol, HDL, or LDL . This is the first study, to our knowledge to characterize the relationships between biomarkers of PFAS exposure and blood lipids in adolescents living in agricultural settings.

We expected a direct association between PFAS and serum lipds but observed an inverse relationship between PFAS and triglycerides only in females. Most adolescent studies that found a direct association were not stratified by sex. Only two other studies in adolescents evaluated effect modification by sex on the association between PFAS and triglycerides. One cross-sectional study of 877 adolescents conducted in the US found an inverse association between PFOS and triglycerides in an unadjusted model for both males and females. However, no association was observed when adjusted for sex and age (Geiger et al., 2014). Another cross-sectional study of 12,476 children and adolescents found PFOS to be inversely associated with triglycerides in adolescent females but not males when stratified by sex (Frisbee et al., 2010). In one case-control study that did not stratify by sex, researchers found an inverse relationship between PFOS and triglycerides (Predieri et al., 2015). Other adolescent studies that were not stratified by sex found a direct association between PFAS and triglycerides in Denmark, US, and Taiwan, respectively (Timmermann et al., 2014; Koshy et al., 2017; Zeng et al., 2015). Furthermore, no research has been conducted on this topic in Latin America or in an agricultural setting to our knowledge.

Our findings on other blood lipids were consistent with few adolescent studies that

stratified the data by sex. In one cross-sectional study on Korean adolescents, the findings showed no associations between PFOS, PFOA, and PFNA with total cholesterol, HDL, and LDL. However, the results showed no inverse association with the three PFAS and triglycerides (Kang et al., 2018). Other studies that did not stratify the data by sex, found no association between PFOS and PFOA with total cholesterol and HDL (Abraham et al., 2020; Predieri et al., 2015). Contrary to our findings, most adolescent studies found a direct trend between PFOA and PFOS with total cholesterol and LDL in sex-adjusted models (Geiger et al., 2014; Frisbee et al., 2010; Averina et al., 2021; Koshy et al., 2017; Zeng et al., 2015).

Our findings were consistent with a few adult studies; however, the results in adult studies are mixed. One cross-sectional study conducted on a highly exposed population in Nunavik, found an inverse association between PFOS and triglycerides only in females after stratifying the data by sex (Château-Degat et al., 2010). Another cross-sectional study in the US investigated the sex and obesity differences in the relationship between PFAS and serum lipid concentrations. Researchers found an inverse association between triglyceride and PFOS and PFHxS among obese and non-obese males and females (Jain & Ducatman, 2019). Although not stratified by sex, a Swedish nested case-control study on 187 adults also found an inverse trend between PFOA, PFOS, PFNA, and PFHxS with triglycerides (Donat-Vargas et al., 2019). However, similar to adolescent studies, many epidemiological adult studies that stratified the data by sex demonstrated direct trends between PFAS with LDL and total cholesterol, but no association with triglycerides (Canova et al., 2020; Batzella et al., 2022; Bjorke-Monsen et al., 2020; Starling et al., 2019).

Several animal studies also reported associations between PFAS and triglycerides; however, results in animal studies have mixed results (Nakagawa et al., 2012; Das et al., 2017;

Butenhoff et al., 2002). Consistent with our results, a few rodent-model studies found PFAS concentrations to be inversely associated with lower levels of triglycerides (Pouwer et al., 2019; Loveless et al., 2008; Yan et al., 2014). However, contrasting our findings, one study on mice found a stronger direct relationship between PFOA concentrations and serum triglycerides in females than in males (Schlezinger et al., 2021), whereas other studies reported no significant association between PFAS and serum triglycerides (National Toxicology Program, 2019; Nakamura et al., 2009).

The observed inverse associations between serum PFAS and triglycerides among females in this study suggests a sex-specific hypolipidemic effect of PFAS. While lower triglycerides are generally perceived as beneficial, it is crucial to consider their potential impact on essential organ functions during adolescence, where critically low triglyceride levels can adversely affect growth and development (Miller et al., 2011; National Research Council (US) Committee on Diet and Health, 1989; Zhao et al., 2021). It is also necessary to take into consideration the adverse health risks associated with increased PFAS levels, such as heightened susceptibility to kidney or testicular cancer, high blood pressure, and reproductive and developmental issues (U.S. Environmental Protection Agency, 2023).

Our findings may have differed from other studies due to several factors. Firstly, most studies assessing PFAS-exposed populations have reported higher serum concentrations compared to those of our study. More specifically, the average PFAS concentrations in adolescents, ages 12-19 years old, from the National Health and Nutrition Examination Survey (NHANES) 2011-2018 were greater than that of ESPINA. The PFOS and PFNA concentrations from NHANES 2011-2018 was nearly six times greater than that of our study population. The PFOA concentrations from NHANES 2011-2018 was approximately 5 times greater than that of

the ESPINA participants (Centers for Disease Control and Prevention, 2022d).

PFAS may alter triglyceride concentrations in the human body by disrupting hepatic lipid metabolism (Tan et al., 2013; Jain & Ducatman, 2019; Arvind et al., 2019). Furthermore, PFAS may influence lipid regulation-associated genes like the microsomal triglyceride transfer protein (MTTP) gene (Marques et al., 2022) or affect the expression of the lipoprotein lipase (LPL) gene responsible for producing LPL, which breaks down triglycerides from lipoproteins (Wang and Eckel, 2009).

It is unclear why an effect modification by sex was observed in this study. A possible explanation could be that females had lower PFAS concentrations than males which could be suggestive that at lower concentrations, PFAS may decrease triglyceride concentrations, but not at higher concentrations. Further investigation is needed to understand this phenomenon. The sex difference in serum PFAS concentrations could be due to females' unique physiological processes, such as menstruation and lactation, which may allow them to eliminate PFAS more efficiently than males (Kingsley et al., 2018; Lorber et al., 2015; Wong et al., 2014). Hormonal and genetic sex differences play a pivotal role in lipoprotein metabolism (Palmisano et al., 2018). Estrogen, a hormone predominantly present in females, has been shown to have a beneficial impact in lipoprotein metabolism by increasing HDL levels, which helps remove cholesterol from the bloodstream (Lamon-Fava et al., 2006). This information suggests that females may have a better triglyceride metabolism than males. Additionally, genetic variations in genes responsible for lipid transport, metabolism, and synthesis can influence the composition and distribution of lipoproteins in the blood. These genetic differences can impact how lipids are processed and transported through the bloodstream (Paththinige et al., 2017). Conducting a comprehensive investigation into the sex-based distinctions in liver enzymes, hepatic lipid

metabolism, and cholesterol biomarkers might provide some clarity behind the effect modification by sex observed.

There were several strengths to this study. This is one of only a few studies that examined adolescents exposed to PFAS, investigated an association with triglycerides, and disaggregated that data by sex. Furthermore, this is the first study to assess this relationship among adolescents in Latin America and in an agricultural setting. Most adult and adolescent studies of PFAS in high-risk populations describe exposure through contaminated water (Sunderland et al., 2018). However, we suspect a source of PFAS exposure in our sample population may be from PFAS present in pesticides, as described above (Suarez-Lopez et al., 2012). Further examination of PFAS exposure is needed to understand other sources and routes of exposure.

Important limitations of this study include a small sample size and a cross-sectional study design, which precludes us from understanding the long-term effects of PFAS exposure, as well as the directionality of the association; that is, whether PFAS affect triglyceride concentrations or vice versa. Moreover, the study findings are not necessarily generalizable to the population of Pedro Moncayo. Although the full ESPINA cohort is likely reflective of the population of Pedro Moncayo, the subset of participants included in the present pilot study comprised participants with both high and low potential of pesticide exposures. Finally, there may be confounding variables that were not controlled for, which affects the strength of the associations that were assessed. Future studies should control for fasting status, caloric intake, diet, and preexisting health conditions that impact lipid levels, such as fatty liver disease (Zuanna et al. 2021).

Our findings highlight the need for further research concerning the sex-specific associations of PFAS on serum lipids. Further research in these areas is important for developing preventative measures to reduce different populations' exposure to PFAS.

Conclusion

Our findings are indicative of an inverse association between triglycerides and PFOS, PFOA, and PFNA in adolescent females, but not males. Further research assessing effect modification by sex, as well as potential mechanisms for this sex difference is needed to better understand the implications of this association on human health. This study expands the limited research currently available on the prevalence and health effects of PFAS in Latin American and rural populations, and we highlight the need for more research on these populations.

Guerra, Michelle was the primary author of this thesis. Coauthors Kayser; Georgia, Checkoway, Harvey; and Suarez-Lopez, Jose Ricardo contributed to idea formulation for all chapters and provided comments and edits on multiple drafts of this thesis.

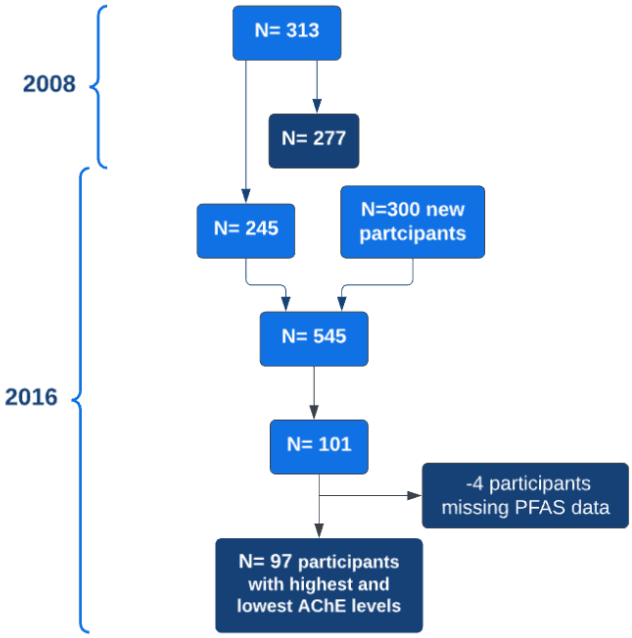

Figure 1: Participant flow chart.

Table 1: Participant characteristics by tertiles of PFOS.

	Total	**PFOS Activity Tertiles**			
PFOS Range, U/mL	**12-71**	**12- 43**	**44- 65**	**66- 71**	**P-trend**
N	**97**	**31**	**34**	**32**	
Age, years	14.67 (1.65)	14.51 (1.73)	14.95 (1.63)	14.55 (1.61)	0.516
Gender (male), %	54	35	59	66	0.035
Height-for-age, z-score	−1.43 (0.94)	−1.54 (1.07)	−1.35 (0.93)	−1.42 (0.84)	0.837
BMI-for-age, z-score	0.26 (0.87)	0.41 (0.70)	0.23 (0.91)	0.15 (0.96)	0.109
AChE, U/mL	3.80 (0.64)	3.64 (0.54)	3.87 (0.69)	3.90 (0.66)	0.077
Hemoglobin, mg/dL	13.04 (1.18)	12.77 (1.03)	13.15 (1.26)	13.17 (1.22)	0.414

***The displayed values are either percentages or mean (SD).** Abbreviations: N, number of participants; AChE, acetylcholine level; PFOS, perfluorooctanesulfonic acid; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid. P-values were calculated using a generalized linear model using a log-transformed continuous variable for PFOS concentration.

Table 2: Average serum PFAS concentrations among the total number and sex-stratified adolescent population.

	Total		Females		Males	
PFAS	**N**	**Geometric Mean (95% CI)**	**N**	**Geometric Mean (95% CI)**	**N**	**Geometric Mean (95% CI)**
PFOS, ng/mL	97	0.57 (0.52, 0.63)	45	0.51 (0.44, 0.60)	52	0.63 (0.56, 0.70)
PFOA, ng/mL	97	0.33 (0.31, 0.36)	45	0.29 (0.26, 0.33)	52	0.37 (0.34,0.41)
PFNA, ng/mL	97	0.09 (0.08, 0.10)	45	0.08 (0.07, 0.09)	52	0.10 (0.09, 0.11)

* Abbreviations: PFOS, perfluorooctanesulfonic acid; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid.

Table 3: Associations between PFAS and serum lipids in adolescents.

	Percent difference of lipids per 50% increase in PFAS concentration (95% CI)			
	Triglycerides	**Total Cholesterol**	**HDLc**	**LDLc**
PFOS	-5.89 (-13.21, 2.04)	1.80 (-2.83, 6.65)	1.32 (-2.09, 4.86)	1.61 (-2.94, 6.38)
PFOA	**-10.86 (-20.14, -0.51)**	-0.29 (-5.97, 5.72)	0.86 (-3.41, 5.31)	1.86 (-4.63, 8.79)
PFNA	-2.86 (-13.97, 9.68)	0.93 (-5.06, 7.31)[a]	1.34 (-3.36, 6.27)	-2.67 (-9.52, 4.69)

*Displayed values are percentages. Abbreviations: PFOS, perfluorooctanesulfonic acid; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid. Adjusted for age, sex, height-for-age, BMI-for-age, AChE activity, and hemoglobin concentration.

[a] p-value for quadratic term = 0.008, β= 141.8, β^2 = -1055.0

Table 4: Percent difference in triglyceride concentration per 50% increase in PFAS when stratified by sex.

	Percent difference in triglyceride concentration per 50% increase in PFAS (95%CI)	
	Females	**Males**
PFOS	**-15.01 (-24.72, -4.06)**	6.12 (-5.09, 18.65)
PFOA	**-16.55 (-28.16, -3.07)**	0.61 (-11.07, 13.82)
PFNA	**-25.49 (-36.93, -12.00)**	10.51 (-1.09, 23.46)

*Displayed values are stratified by sex and are either percent or mean (SD). Abbreviations: 95% CI, 95% confidence interval; PFOS, perfluorooctanesulfonic acid; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid.

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