COLLAGEN REMODELING PROTEINS, INFLAMMATORY BIOMARKERS, AND FABP REGULATION IN UNDERSTANDING THE PATHOGENESIS OF ATRIAL FIBRILLATION

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BACKGROUND INFORMATION

- Atrial fibrillation is the most common form of cardiac arrythmia seen in patients globally with around 3 million active cases.
- Atrial fibrosis is the process by which collagen and other extracellular matrix are deposited within the atria. Certain abnormalities in the structure, structural remodeling with collagen remodeling proteins, can cause atrial fibrosis. Atrial fibrosis has been said to have an association or correlation with atrial fibrillation from previous studies.

BACKGROUND INFORMATION

- Fatty acid binding proteins are a family of proteins with attraction for long chain fatty acids and fat-soluble molecules.
- Long chain fatty acids (LCFAs), in excess, can lead to lipotoxicity which increases the risk of a potential arrythmia. Fatty Acid Binding Proteins (FABPs) bind to LCFAs to create a lower level of these fatty acid chains in the cytoplasm. Increased LCFAs has a direct correlation with increased FABPs which can ultimately increase the risk of arrythmia.

PURPOSE

- The purpose of the study was to analyze levels of collagen remodeling proteins, specifically PINP and PICP, and L-FABP in an AF cohort to determine whether they can serve as viable biomarkers in the management and diagnosis of AF.
- We assume there will be an upregulation of collagen remodeling proteins, inflammatory biomarkers, and FABPs in the AF patients as compared to the control cohort.

SPECIFIC AIMS

- The purpose of the study was to analyze levels of collagen remodeling proteins, specifically PINP and PICP, and L-FABP in an AF cohort to determine whether they can serve as viable biomarkers in the management and diagnosis of AF.
- We assume there will be an upregulation of collagen remodeling proteins, inflammatory biomarkers, and FABPs in the AF patients as compared to the control cohort.

MATERIALS AND METHOD

- The blood of the AF patients was collected, after receiving approval from the IRB protocol, and centrifuged. The blood was aliquoted and stored at –80 degrees C. The samples were blindly analyzed in the Hemostasis and Thrombosis Research laboratories. Additional 50 control samples were collected from a commercial vendor (George King Biomedical).
- Commercially available sandwich ELISA kits will be used to quantify these biomarkers in patients with AF and the control cohort.

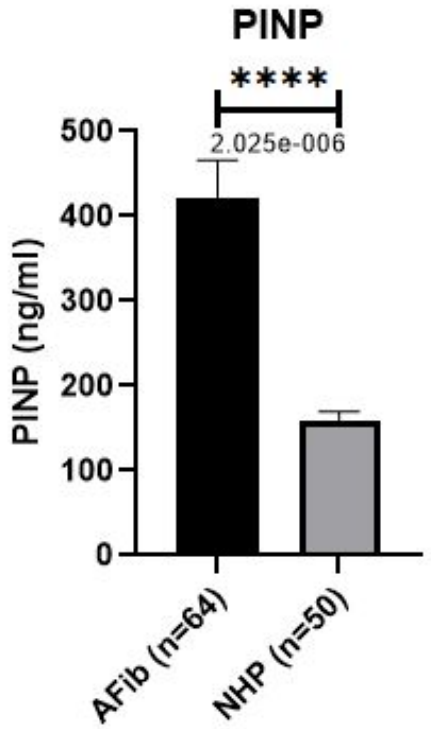
RESULTS

PINP, PICP, AND FABP

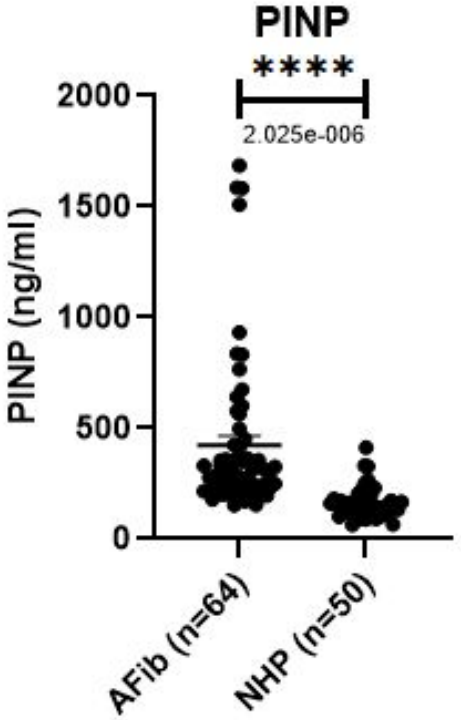
PINP

- It can be present as four different forms including intact trimeric form, dimeric form, monomeric form, and fragmented form.
- Procollagen type I propeptides are derived from collagen type I, which is the most common collagen type found in mineralized bone.
- PINP exhibits diurnal variation, with higher values occurring at night. PINP is metabolized in the liver. Individuals with severe liver disease may have elevated PINP levels.

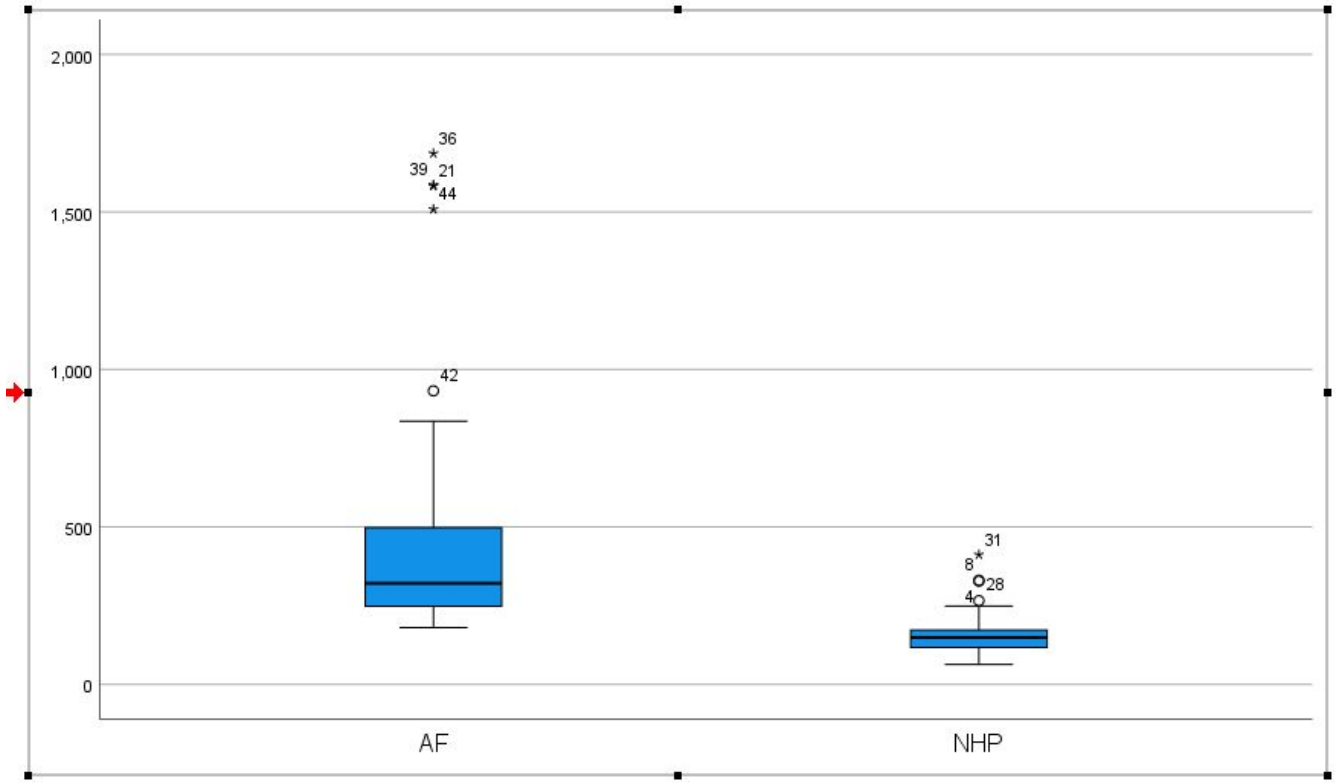
PINP GRAPHS AND RAW DATA



PINP	AF (n=64)	NHP (n=50)
Mean ± SEM	422.32 ± 43.78	159.33 ± 9.61
STD DEV	350.265	67.9046
Minimum	153.17	63.65
Maximum	1685.5	412.3
P-Value	<0.0001	
NHP	165% increase of mean	

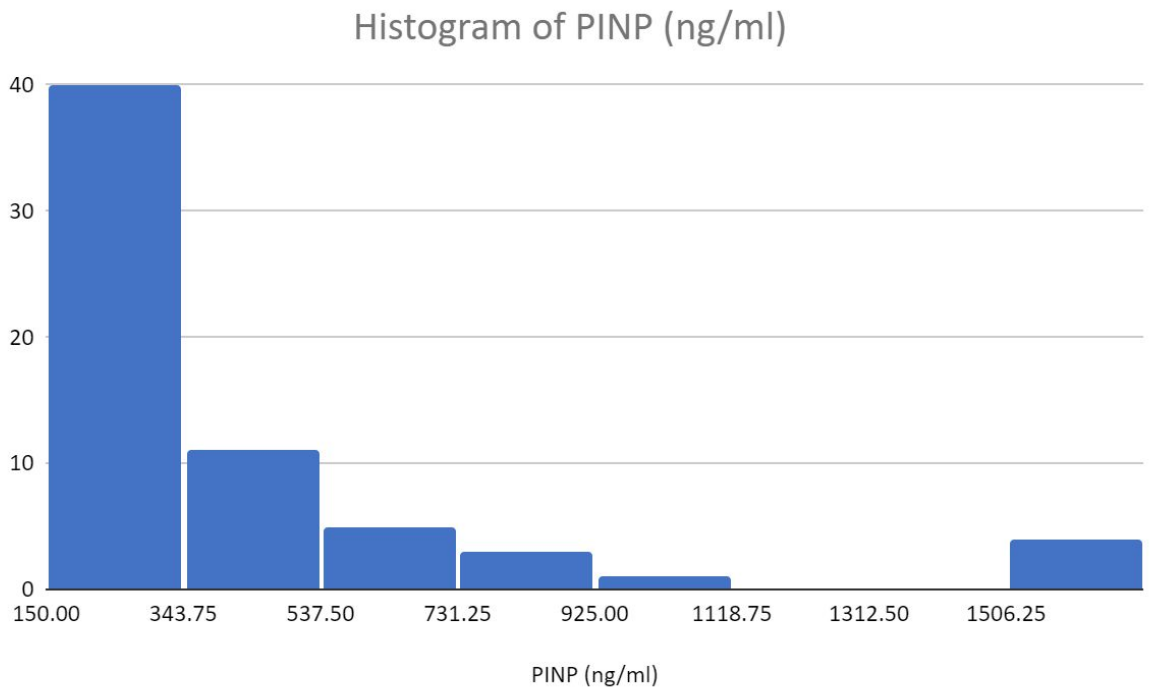


BOX PLOT AND QUARTILE ANALYSIS



N	Valid	64	50
	Missing	0	14
Median		305.1500	149.2450
Minimum		153.17	63.65
Maximum		1685.85	412.13
Percentiles	10	195.1800	91.4960
	20	216.2000	109.7160
	25	229.1000	117.4150
	30	246.8950	118.8330
	40	273.7500	130.5800
	50	305.1500	149.2450
	60	331.3500	161.6760
	70	360.9850	171.9070
	75	421.4775	174.7200
	80	564.0100	197.3140
	90	833.9900	247.6480

SKEWNESS AND FREQUENCY DISTRIBUTION



		A	B
		AFib (n=64)	NHP (n=50)
1	Number of values	64	50
2			
3	Minimum	153.2	63.65
4	25% Percentile	229.1	117.4
5	Median	305.2	149.2
6	75% Percentile	421.5	174.7
7	Maximum	1686	412.1
8	Range	1533	348.5
9			
10	Mean	422.3	159.9
11	Std. Deviation	350.3	67.94
12	Std. Error of Mear	43.78	9.608
13			
14	Skewness	2.486	1.664
15	Kurtosis	5.885	3.605
16			
17			
18			
19			
20			

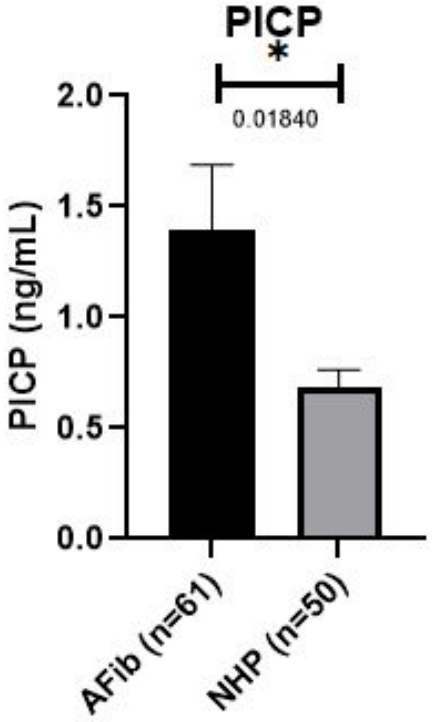
CONCLUSION

- There was evidence of an upregulation of the PINP biomarker which matches the expected results. This was a significant increase of about 165%.
- There was a significant outlier at 1685.5 which significantly increased the mean. In addition to this outlier, there were four other outliers which represented much of the increase in the mean. The data is statistically significant which means that the there is a definite correlation between the elevated levels of PINP and the NHP group.

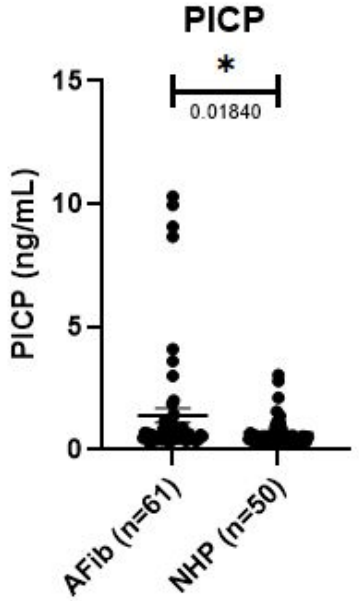
PICP

- There is a direct correlation between bone collagen synthesis and bone formation rate. PICP may be an important determinant of bone formation.
- The elevated levels of PICP directly relates to a higher risk of suffering from atrial fibrillation as concluded in the study by Javier Diez. Elevated levels of PICP are also consistent with LA Fibrosis.
- PICP correlates with left atrial myocardial interstitial fibrosis which perpetuates atrial fibrillation. The deposition of type 1 collagen in the left atrium of the heart causes this type of fibrosis.

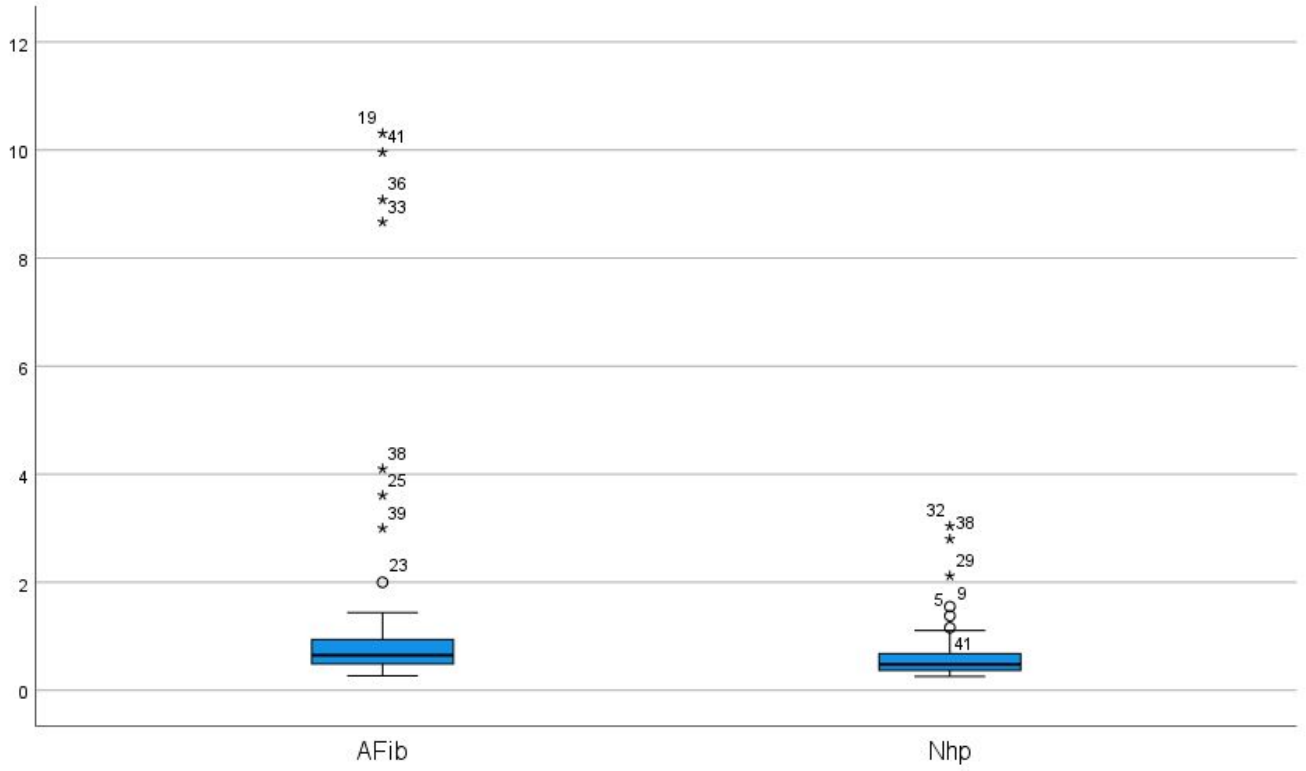
PICP GRAPHS AND RAW DATA



	AF (n=61)	NHP (n=50)
Mean	1.395902	0.6814
STD. Dev	2.287188	0.5846646
SEM	0.178727	0.0963645
Minimum	0.27	0.26
Maximum	10.31	3.04
% change	104.86% increase	



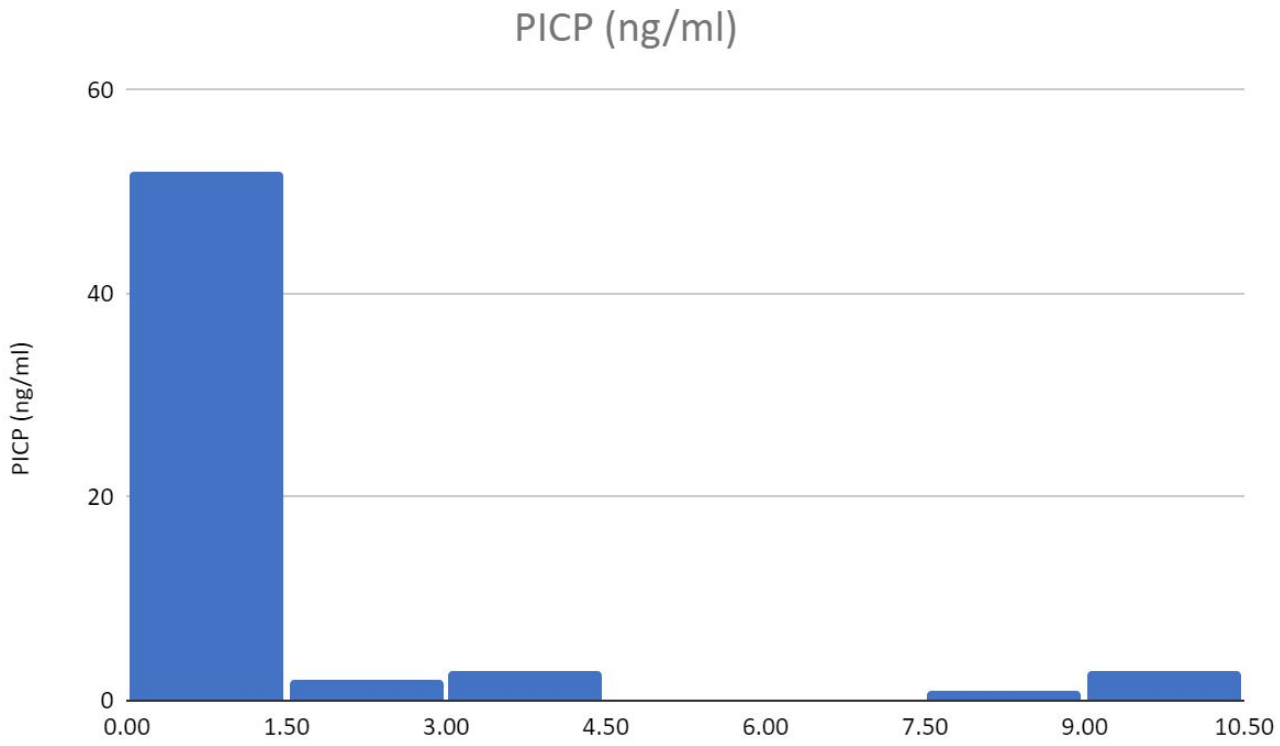
BOX PLOTS AND QUARTILE ANALYSIS



Statistics

		AFib	Nhp
N	Valid	61	50
	Missing	0	11
Minimum		.27	.26
Maximum		10.31	3.04
Percentiles	10	.4020	.3020
	20	.4600	.3500
	25	.4850	.3675
	30	.4900	.3950
	40	.5300	.4500
	50	.6100	.4800
	60	.7060	.5360
	70	.8140	.5910
	75	.8950	.6825
	80	1.0800	.8360
	90	3.4880	1.3580

SKEWNESS ANALYSIS AND FREQUENCY DISTRIBUTION



Descriptives

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Skewness	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error
AFib	61	.27	10.31	1.3959	2.28719	3.140	.306
Nhp	50	.26	3.04	.6814	.58466	2.767	.337
Valid N (listwise)	50						

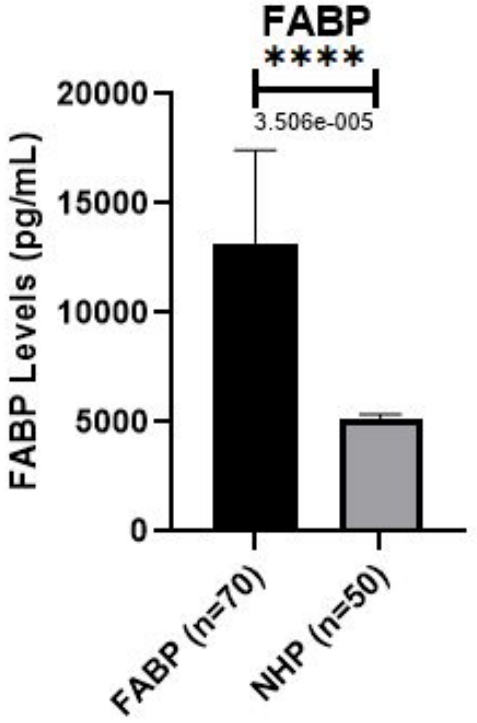
CONCLUSION

- There is an evident increase in the PICP levels when comparing Atrial Fibrillation patients and the NHP group. The increase is of about 104.86%. When analysis of the AF patients, there is evidence of four major outliers in the data which are 8.67, 9.08, 9.96, and 10.31. These outliers are far greater than other values and may have caused a large increase in the AF samples.
- The data is statistically significant. We had a good number of samples to draw a strong conclusion. The % change for PICP was around 60% less than the % change in the PINP biomarker.

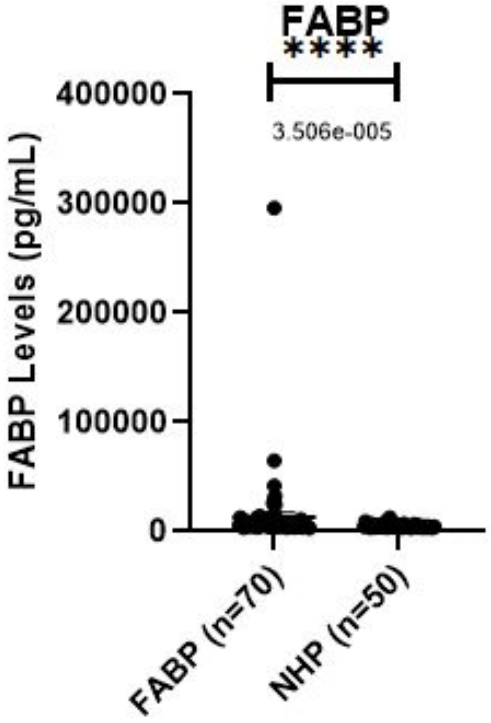
FABP

- They are important for intracellular transport and cellular fatty acid metabolism as they reversibly bind and transport long-chain polyunsaturated fatty acids from cell membranes to the mitochondria. They also play as cytoprotectants to fatty acid oxidative stressors.
- Liver Fatty Acid Binding Protein also known as FABP1 comprises 2-11% of the cytosolic protein of normal hepatocytes. It is also found in tubular kidney cells, the alveolar epithelial of the lung, and in enterocytes of the small intestine.

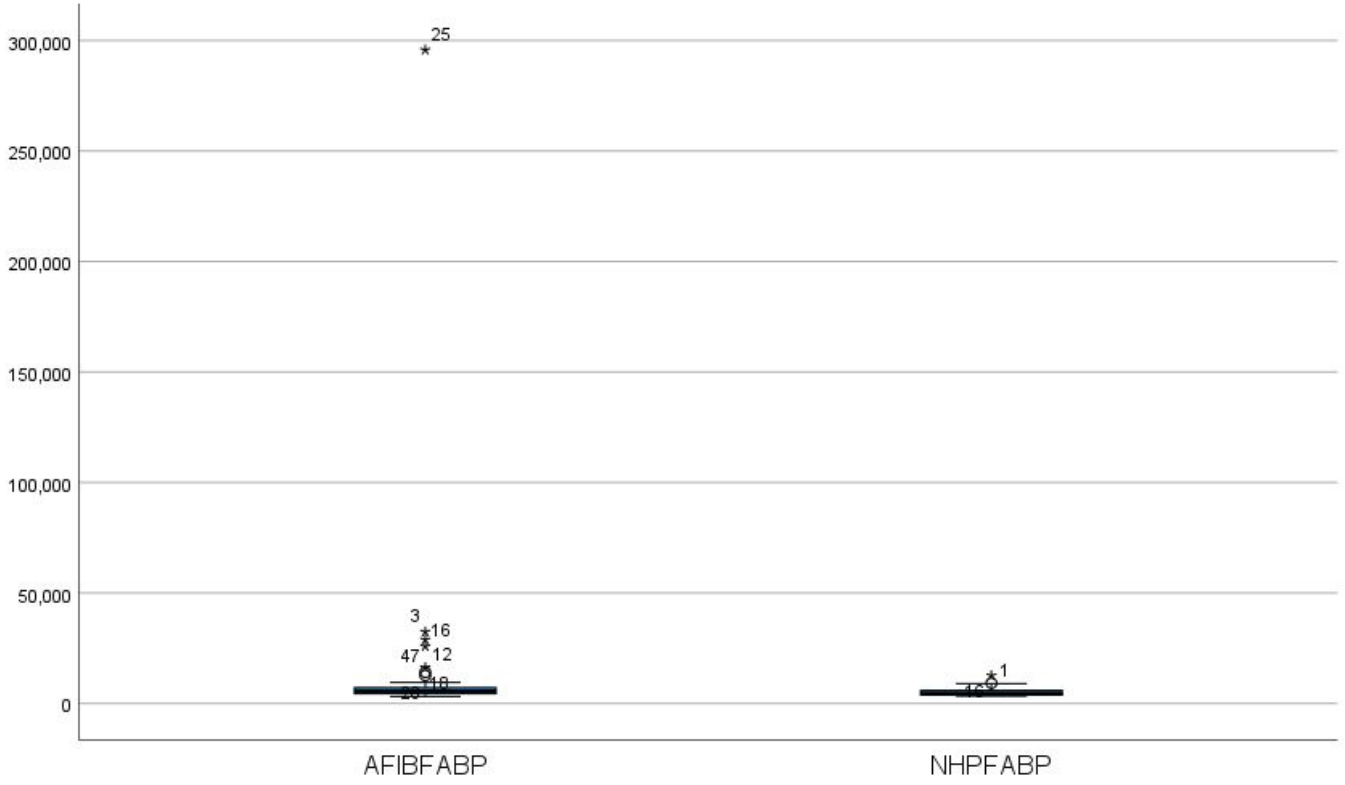
FABP GRAPHS AND RAW DATA



	AF (n=70)	NHP (n=50)
Mean	13186.7517	5116.3294
STD. Dev	35616.30483	1767.88278
SEM	4256.96265	250.01638
Minimum	3203.62	3408.44
Maximum	295778.86	12670.86
% change	157.75% increase	



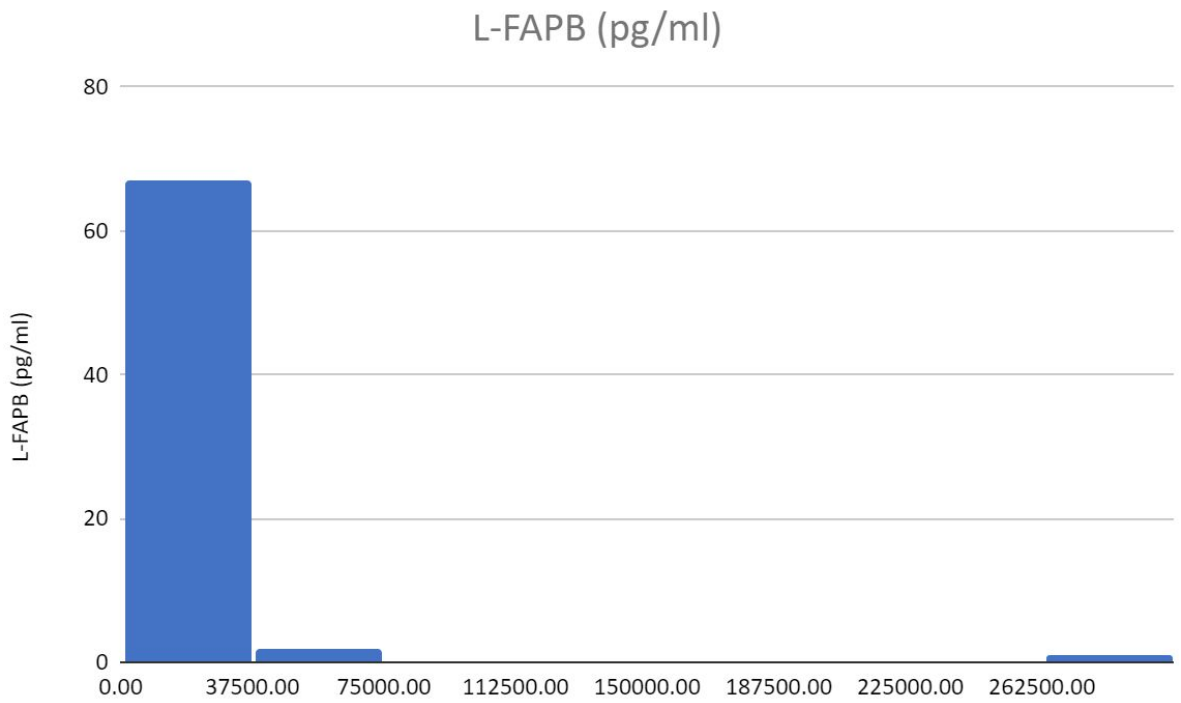
BOX PLOT AND QUARTILE ANALYSIS



Statistics

		AFIBFABP	NHPFABP
N	Valid	70	50
	Missing	0	20
Std. Error of Mean		4256.96265	250.01638
Median		5966.1350	4625.1900
Std. Deviation		35616.30483	1767.88278
Minimum		3203.62	3408.44
Maximum		295778.86	12670.86
Percentiles	10	4060.4920	3557.0800
	20	4493.6880	3743.5680
	25	4841.2775	3833.0800
	30	4929.2370	3971.8090
	40	5428.2140	4323.0560
	50	5966.1350	4625.1900
	60	6769.4260	5022.7620
	70	7929.6530	5625.2440
	75	9062.5600	5926.5600
	80	9551.1380	5933.9300
	90	23830.8810	7234.7690

SKEWNESS AND FREQUENCY DISTRIBUTION



Descriptives

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Skewness	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error
AFIBFABP	70	3203.62	295778.86	13186.7517	35616.30483	7.502	.287
NHPFABP	50	3408.44	12670.86	5116.3294	1767.88278	2.136	.337
Valid N (listwise)	50						

Descriptives

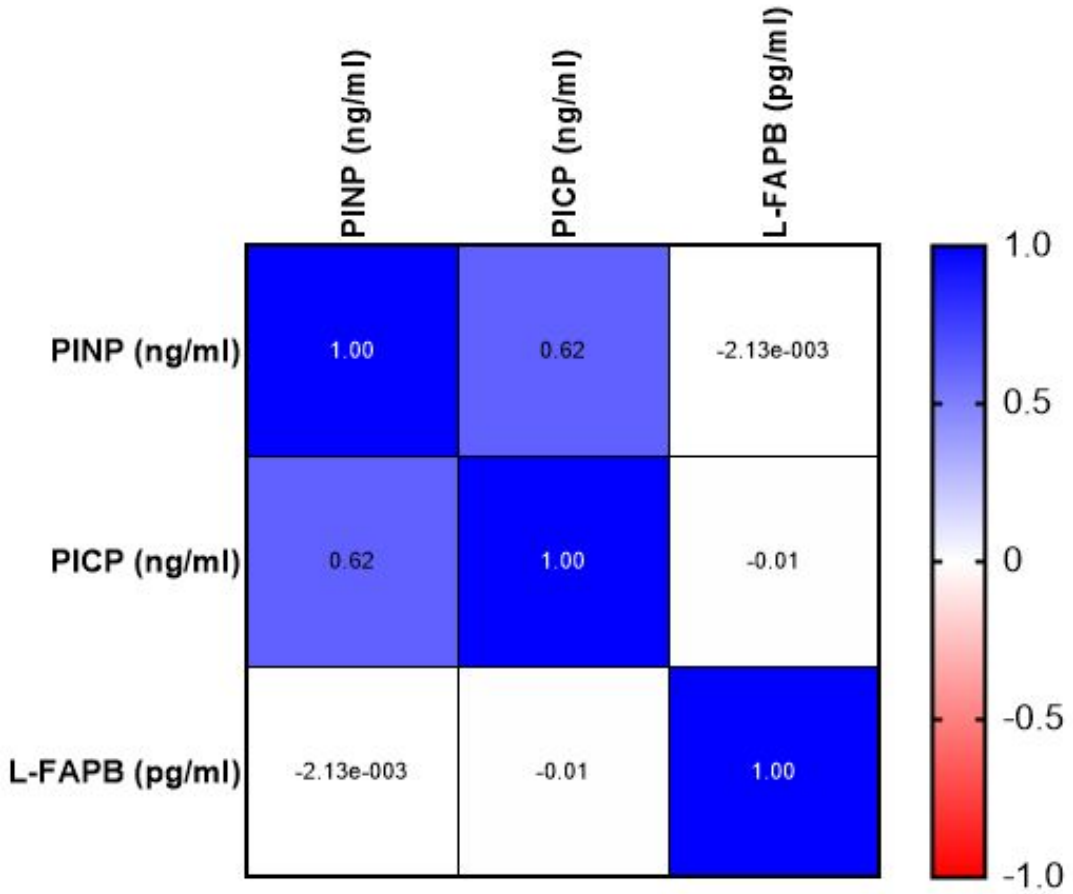
Descriptive Statistics

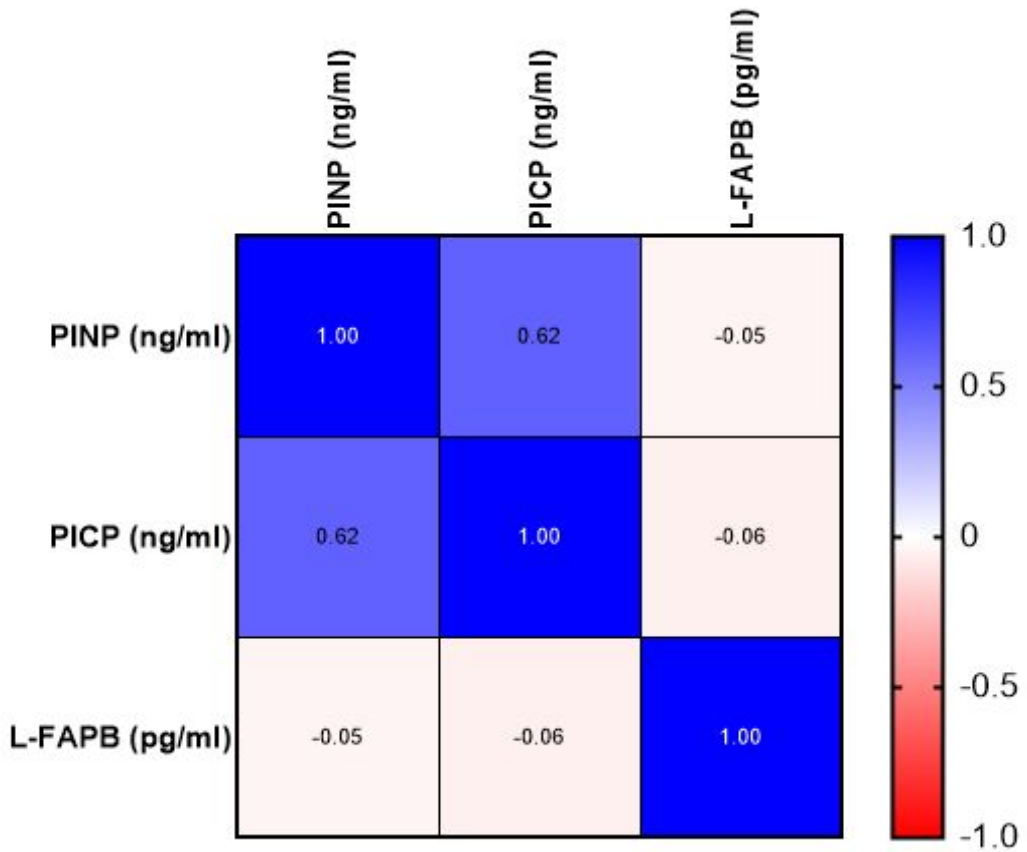
	N	Minimum	Maximum	Mean	Std. Deviation	Skewness	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error
AFIBFABP	69	3203.62	64918.05	9091.2139	9786.16548	3.795	.289
NHPFABP	50	3408.44	12670.86	5116.3294	1767.88278	2.136	.337
Valid N (listwise)	49						

CONCLUSION

- There is an evident upregulation of L-FABP in AF patients as compared to the NHP patients with a percent change 157.75%. The data is heavily condensed from the 0% to 80% as compared to the significant increase that occurs to the 90%. There is one outlier, and the data is relatively low varied.
- The data is statistically significant to four digits meaning that there is an evident upregulation of the FABP biomarker in AF patients and NHP patients, but not to the level demonstrated by the mean. Without the outlier, the percent change is 77.69%.
- The distribution was not normally skewed, so I used a unpaired T test, specifically the Mann-Whitney Test, to represent the data.

CORRELATION ANALYSIS





FINAL CONCLUSION

- **The results from the data collected for the PINP, PICP, and FABP levels in the AF cohort demonstrate an evident upregulation as compared to the respective levels of these biomarkers in the NHP.**
- **The outlier for the FABP group was significant but did not change the outcome of the data as the data remained statistically significant and still experienced 77.69% change of the mean without the outlier.**

FUTURE PLANS

- **Complete an abstract for FASEB**
- **Conduct correlation analyses on multiple biomarkers to add to a possible presentation in October**

Thank You

Preparing people to lead extraordinary lives