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Running title: Cardiac histopathology and miRNAs in New Zealand Pacific Islanders

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1. **Department of Physiology, School of Biomedical Sciences, University of Otago, Dunedin, New Zealand**
2. **Department of Medicine, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand**
3. **HeartOtago, University of Otago, Dunedin, New Zealand**

***Corresponding author**: regis.lamberts@otago.ac.nz or andrew.bahn@otago.ac.nz

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ABSTRACT:
Cardiovascular disease (CVD) is the leading cause of death worldwide and in New Zealand. However, a significant inequality in the burden of CVD amongst different ethnic groups exists with a 2 - 3-times higher CVD mortality rate in Pasifika compared to Pākehā. It is unknown whether a difference in cardiac fibrosis might underly this ethnic inequality in CVD mortality. To address this, we determined cardiac fibrosis, myocardial fat infiltration, and the expression of some key miRNAs (miR-15a, miR-15b, miR-34a and miR-153) in right atrial appendages of Pacific Islanders and New Zealand European patients (n=21) undergoing cardiopulmonary bypass surgery. Cardiac fibrosis was measured by total collagen deposition identified by Picro Sirius Red staining, whereas fat accumulation was determined via Oil-Red-O staining. No differences in cardiac fibrosis were observed between ethnic groups (Collagen: Pasifika 23.4±12.5% vs. New Zealand European 29.4±13.2%, one-way ANOVA, p=0.17). Similarly, no differences were observed in accumulation of lipid nor the expression of the miRNAs examined (-15a, -15b, -34a and -153) between different groups. In conclusion, the earlier requirements for surgical intervention for CVD of Pasifika in Aotearoa might not be explained by differences in miRNAs associated with cardiomyocyte loss, fibrosis or myocardial lipid infiltration.

New and Noteworthy

Despite the established significant inequality in the burden of CVD amongst the Pasifika compared to the Pākehā (New Zealand European) populations in Aotearoa, we found no difference in histopathological (cardiac fibrosis, lipid infiltration, or associated pro- or anti-fibrotic miRNAs) features of right atrial tissue from cardiac surgery patients of the different ethnic groups.

KEYWORDS: Cardiac fibrosis, microRNA, fat infiltration, human cardiac tissue, health inequality

INTRODUCTION:

Cardiovascular disease (CVD) is the major cause of death worldwide [1] and accounts for approximately 35% of deaths within Aotearoa (New Zealand) [2]. The risk, morbidity and mortality consequences of CVD are markedly varied in different races and ethnic backgrounds [3]. Indigenous populations such as American Indians, Alaska natives and Aboriginal Australians are often shown to be at higher risk of CVD [4]. This is often associated with socioeconomic status [5] alongside elevated incidence of classical CVD risk factors such as hypertension, obesity, diabetes and high cholesterol [6]. This inequality in the burden of CVD is similar in Pasifika and Māori people in Aotearoa who are more likely to have CVD risk factors [7-9] and have a 2 – 3-times higher mortality rate than Pākehā people of NZ European descent [10]. The ethnic disparity in CVD risk factors has been extensively studied [11-12] however; potential underlying physiological differences behind the difference in CVD incidence are not clearly understood.

Cardiac fibrosis is a hallmark of cardiac remodelling resulting from excess accumulation of extracellular matrix (ECM) components in the interstitial and perivascular regions of the heart, which eventually contributes to impaired cardiac function [13]. However, the complex molecular mechanisms that control cardiac fibrosis are not fully understood. One potential mechanism is alteration to the microRNA (miRNA) profile. MiRNAs are small non-coding RNAs that negatively modulate the translation of their target proteins. The overall profile of miRNAs is known to change in disease [14], including CVD [15]. Numerous miRNAs have been implicated in fibrosis, and down regulation of the antifibrotic miRNA-15a and miRNA-15b have been associated with several cardiac diseases [16-17]. Cardiac fibrosis often occurs as a consequence of cardiomyocytes loss, and this has been associated with the upregulation of miRNA-34a both in age [18] and diabetes [19] and miRNA-153 in cardiac disease [20-21]. Alternatively, myocardial lipid infiltration has also been associated with an unfavorable change in cardiac structure and function [22-23].

In this study, we aimed to investigate the differences in cardiac fibrosis, myocardial lipid infiltration, and expression of a select group of miRNAs in cardiac tissue from Pasifika and New Zealand Europeans. We hypothesized that cardiac fibrosis and myocardial lipid infiltration in right atrial appendage tissue of patients undergoing coronary artery bypass surgery would be increased in Pasifika compared to Pākehā populations. In addition, the expression profiles of antifibrotic miRNAs (miRNA-15a and -15b) would be lower and apoptotic miRNAs would be higher in cardiac tissue of Pasifika.

METHODOLOGY:

Ethics:

The study was conducted under human ethical approval granted by the Southern Health and Disability Ethics Committee (LRS/12/01/001/AM17) and conformed to the Declaration of Helsinki principles, with patients providing informed consent for collection and use of tissue. Consultation was undertaken with the Ngāi Tahu Research Consultation Committee to provide the framework for an appropriate and mandated research consultation process. Based on input from the Pacific communities in the Otago region, culturally-sensitive protocols were followed for acknowledging and respecting tissue donated from our Pasifika volunteers for inclusion in this study [24].

Inclusion criteria:

The HeartOtago tissue collection database contained 851 patient samples collected between 2012 – 2019. Retrospective analysis was performed on a subset of patients (n=7 per group) from the HeartOtago tissue collection (Figure 1). Inclusion criteria were patients undergoing clinically indicated on-pump coronary artery bypass surgery (CABG) in Dunedin Hospital in the period from 2012-2019, with or without valve replacement. The patients were males with a BMI > 30 kg/m^2 and prescribed at least two different classes of CVD medications prior to surgery (Table 1). Females were excluded as the numbers available were insufficient (n=1) and sex differences in cardiac fibrosis are known [25]. To mitigate compounding effects when comparing between Pasifika and New Zealand European the groups were matched across physiological and clinical characteristics. In addition, to account for age-related differences, a second group of NZ European patients (n=7) was included that was matched for all parameters, including age.

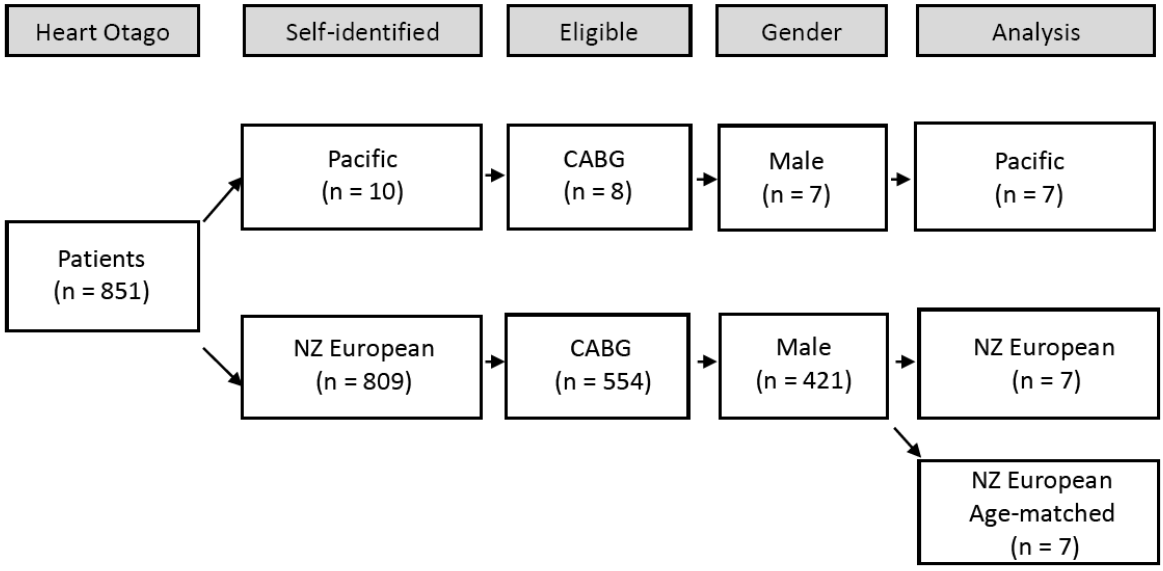
Ethnicity:

Ethnicity was self-reported with selection as outlined in NZ Ministry of Health guidelines [2]. Patients selected had identified as either Pasifika or New Zealand European descent. Within the Pasifika group the patients identified as Samoan (n = 3), Cook Island Māori (n = 3) and Niuean (n =1).

Clinical and anthropometric data:

Standard clinical, biochemical and anthropometric data was collected pre-operatively. Cardiac function was determined from comprehensive echocardiography using commercially available machines (Vivid E9 or E95, GE Healthcare, Chicago, US), according to the recommendations of the American Society of Echocardiography [26].

Figure 1: Patient selection from HeartOtago biobank
CONSORT diagram indicating process of selection of patients from the HeartOtago tissue collection for inclusion within this retrospective study.



Sample preparation:

During cannulation in cardiopulmonary bypass surgery the right atrial appendage (RAA) is routinely removed. This excess tissue was collected and processed for either histological analyses or miRNA assessment. One portion of the RAA was immediately frozen in liquid nitrogen for miRNA assessment, and the remainder part for histological analysis was fixed in 4% neutral buffered formalin (Sigma Aldrich, St Louis, MO, USA) for 24h, prior to treatment with 30% sucrose (Merck, Kenilworth, NJ, USA), and embedding in cryoprotect matrix (Thermoscientific, MA, USA). Frozen sections (8µm) of the RAA were prepared and standard histological stains used to demonstrate key cellular features. The presence of collagen was demonstrated by Picro Sirius Red (PSR) staining, with a light green counterstain [27], whereas fat deposits were identified by Oil-Red-O (ORO) staining [28].

Image analysis:

Digital images of stained PSR sections were obtained with Aperio slide scanner (Leica Biosystems, Wetzlar, Germany). In addition, these sections were imaged at x20 magnification under polarized light, with images captured with a standardized exposure of 208.5s, gain 5.2 and offset -1017. Sections stained with ORO were imaged at x40 on

Moticam 580 digital camera attached to Miotic BA310 light microscope (Motic Asia, Kowloon, Hong Kong).

All image quantification measurements were conducted with the assessor blinded to the patient group. For each patient, images of 15 regions of interest (ROI) were analyzed, taking care to avoid introduction of bias by excluding the edges of each section which could include some pericardial tissue. Quantification of the extent of fibrotic or fat deposition was conducted using a color-based digital analysis method (Adobe Photoshop, CS5 v12.1, San Jose, CA, USA). A standardized mask was created for each color and this mask applied to all sections for quantification.

The color-based quantification system was also applied to the polarized light images of the PSR stained sections. The different color birefringence arises from the different structure of the collagen types: Collagen I show as red/orange color whereas collagen III has yellow/green color [29] . The area of each of the colors was measured separately and then subsequently combined to provide a pixel count of each collagen type. This allowed the calculation of the percentage of collagen I and III the I/III collagen ratio [30].

MiRNA assessment:

Total RNA was isolated from snap frozen RAA samples with miRNeasy mini kit (Qiagen, Hilden, Germany). The primer sequence for the specific miRNAs were: miR-16 (UAGCAGCACGUAAAUAUUGGCG), miR-24 (UGGCUCAGUUCAGCAGGAACAG), miR-15a (UAGCAGCACAUAAUGGUUUGUG), miR-15b (UAGCAGCACAUCAUGGUUUACA), miR-34a (UGGCAGUGUCUUAGCUGGUUGU) and miR-153-5p (UCAUUUUUGUGAUGUUGCAGCU). Quantitative real-time PCR with iTaq Universal SYBR Green Supermix (BioRad, Hercules, CA, USA), was conducted on a StepOne Plus Real Time PCR Systems (Applied Biosystems, Foster City, CA, USA). Individual miRNA expression was normalized to the average of two internal controls (miRNA-16 and miRNA-24) with expression reported as log 2-ΔCt. The inter-plate co-efficient of variation was 4.9%, indicative of reproducibility between PCR runs.

Data analysis:

Data is presented throughout as mean ± SD, with individual patient values shown. Histological values are presented as the average of the 15 ROI, unless specified. Measured differences between ethnic groups were assessed with one-way ANOVA, with Tukey's post-tests. Fisher's exact test was used to demonstrate effective matching in patient clinical characteristics. Correlation between the different components of the RAA (e.g., collagen, fat) and clinical parameters was determined by two-tailed non-parametric Spearman r correlation.

The histopathological composition of the RAA in the patient cohorts was compared by non-parametric one-sample Wilcoxon test to percentage values of collagen, fat and cardiomyocyte expected in healthy individuals with values obtained from the literature as we had no access to cardiac tissue from non-cardiac patients. Normal healthy values were taken from post-mortem studies of individuals aged (50 – 70 years old) of European descent, with no signs of cardiac disease [31-34]. Normal comparator values selected were: 5% for collagen [31], 1% for fat [32], 65% for cardiomyocytes [33] and 0.6 for the ratio of collagen I/III [34].

RESULTS:

The HeartOtago database was investigated to identify patients for inclusion in this retrospective study (Figure 1). Within each self-identified ethnic cohort, males dominated the patient population (>75% samples from males), and 70% of the surgical interventions conducted were coronary artery bypass grafts (CABG). The number of Pasifika CABG patients available within the database was low (n = 8), with only a single female. As sex differences in fibrosis are known [25], the female patient was excluded. The available Pasifika male group was the driving force for the selection of patients from NZ European cohorts. The patients (n=7 per group) were selected to match in terms of obesity, diabetes, hypertension and current CVD medication status. No significant differences in clinical or cardiovascular characteristics were observed between the groups (Table 1). The only major finding was that Pasifika patients were undergoing surgical intervention at significantly ($p = 0.004$) younger ages than NZ European, with CABG performed at 56 ± 11 years old, compared to 79 ± 5 year of age in NZ European patients.

Cardiac fibrosis was demonstrated in all samples by PSR (Figure 2A). RAA collagen fraction was increased in patients undergoing CABG, when compared to published expected values (~5 %) found in healthy right atrial tissue [31], with 23.4 ± 12.5%, 36.7 ± 12.2% and 29.4 ± 13.2% collagen in Pasifika, New Zealand European, and NZ European age-matched patients, respectively. Inter-ethnic comparison showed no significant variation in overall collagen content (Figure 2B) within the RAA ($p = 0.17$). The increase in RAA collagen content did not correlate with RA area ($r= -0.134$, $p = 0.60$) or RA length ($r = -0.208$, $p = 0.42$). Examination of PSR images under polarized light provided further information on the differential distribution of structurally different collagens, allowing estimation of both collagen I and collagen III within the samples [29] (Figure 3A). Like total collagen, the values of collagen I and III in RAA samples from CABG patients were higher compared to published healthy values and showed no inter-ethnic variation (data not shown). In contrast, the ratio of

collagen I/III was unchanged in CABG patients compared to expected healthy value [34] (Figure 2C), although large variations were observed in the age-matched NZ European group. Collagen content showed no associations with clinical or cardiac parameters, either as an overall study cohort or within the different ethnicities.

Another key feature of damaged myocardium is fat infiltration as demonstrated by ORO stain (Figure 3A). The proportion of lipid in the CABG patients was higher ($p < 0.05$) compared to the ~1% previously reported in healthy hearts [32] (Figure 3B). There were no significant differences in lipid infiltration between the ethnic groups ($p = 0.80$) with 12.7 ± 9.2%, 15.3 ± 11.9% and 11.3± 11.9% lipid in the Pasifika, NZ European and NZ European age-matched patient cohorts. Within the entire study cohort or within the different ethnicities there was no association of lipid content within the RAA to general clinical and cardiac characteristics, nor to the size of the RAA. The exception was a positive correlation of lipid to blood pressure (Figure 4). There was a significant ($p < 0.05$) positive Spearman r correlation of percentage lipid to systolic ($r = 0.94$, $p = 0.017$; Figure 4A), diastolic ($r = 0.87$, $p = 0.033$; Figure 4B) and mean arterial blood ($r = 0.89$, $p = 0.033$; Figure 4C) in the Pasifika cohort, with no associations observed in the both the NZ European cohorts (r values less than 0.64).

The other main component of the myocardium is cardiomyocytes, which in the healthy heart occupies 65% of the right-side myocardium [33]. The presence of CVD significantly ($p < 0.01$) reduced the area occupied by cardiomyocytes in all ethnicities compared to healthy control levels (Figure 3C), with no ethnic differences.

Overall, the composition of the RAA proportion of lipid, collagen and cardiomyocytes in the different ethnic groups showed no differences (Figure 3D), and is thus, unlikely to be responsible for the large inequality of health outcomes in the indigenous populations.

We have previously found that the RAA expression of anti-fibrotic miRNA-15a and miRNA-15b was down-regulated in NZ European patients undergoing CABG surgery [17], and that miRNA-34a was increased [19]. Therefore, we investigated whether the expression of these miRNAs was changed dependent on the ethnicity of the CABG patient. We found that the expression of miRNA-15a (Figure 5A) -15b (Figure 5B) and miRNA-34a (Figure 5C) was not different across the three groups ($p = 0.41$, 0.75 and 0.16, respectively). Similarly, there was no difference ($p = 0.80$) in the expression of miRNA-153 (Figure 5D). Interestingly, we did detect an age-related difference in the miRNA-34a when restricting the comparison of the NZ European cohorts ($p = 0.023$), which was not present in the other miRNAs.

Table 1: Clinical and cardiovascular characteristics

	Pasifika (n=7)	NZ European (n=7)	NZ European aged matched (n=7)	p value
Age (years)	56 ± 11	79 ± 14*	57 ± 12	0.004
BMI (kg/m2)	33.6 ± 5.2	30.9 ± 3.6	36.3 ± 6.9	0.2697
BSA (m2)	2.1 ± 0.2	2.1 ± 0.2	2.2 ± 0.4	0.445
Medical history				
Hypertension	4 (57%)	6 (86%)	7 (100%)	0.115
Diabetes	3 (43%)	3 (43%)	3 (43%)	0.999
Myocardial infarction	2 (29%)	1 (14%)	2 (29%)	0.769
Smoking (current or ex)	6 (86%)	4 (57%)	4 (57%)	0.424
Pre-operative AF	3 (43%)	0 (0%)	1 (14%)	0.115
Post-operative AF	3 (43%)	1 (14%)	1 (14%)	0.350
Medication				
Statins	6 (86%)	5 (71%)	7 (100%)	0.311
ACE inhibitors	5 (71%)	4 (57%)	3 (43%)	0.558
Beta blockers	6 (86%)	5 (71%)	7 (100%)	0.311
Diuretics	1 (14%)	1 (14%)	0 (0%)	0.575
Anti-coagulants	1 (14%)	2 (28%)	0 (0%)	0.311
Cardiovascular				
MABP (mmHg)	109 ± 32	95 ± 9	87 ± 10	0.196
Ejection fraction (%)	58.6 ± 11.2	52.4 ± 9.8	53.1 ± 4.3	0.417
Fractional shortening (%)	36.6 ± 11.6	34.6 ± 10.3	29.9 ± 8.7	0.587
RA area (cm^2)	18.6 ± 4.8	15.7 ± 4.2	16.6 ± 2.6	0.456
RA length (cm)	5.3 ± 0.6	5.4 ± 0.7	5.3 ± 0.4	0.929

Physical characteristics, medical history, medication and cardiovascular function of patients included in the study (n=7 per group). Patients self-identified as of Pacific Island or NZ European descent. The only statistical difference detected was that Pasifika underwent CABG at younger ages than NZ Europeans (*). BMI, body mass index; BSA, body surface area; AF, atrial fibrillation; ACE, angiotensin-converting-enzyme; MABP, mean arterial blood pressure; RA, right atrial.

Figure 2: Cardiac fibrosis of the right atrial appendages (RAA) of coronary artery bypass graft (CABG) patients. **Fig 2A**: Representative RAA images from each ethnic cohort stained with Picro Sirius Red (PSR) under bright field and polarized light. In PSR stained sections collagen is stained red (yellow arrows) and cardiomyocytes stained green. Collagen shows birefringence under polarized light, with orange/red birefringence depicts collagen type I whereas yellow/green demonstrates collagen type III. Scale bars represent 100µm.

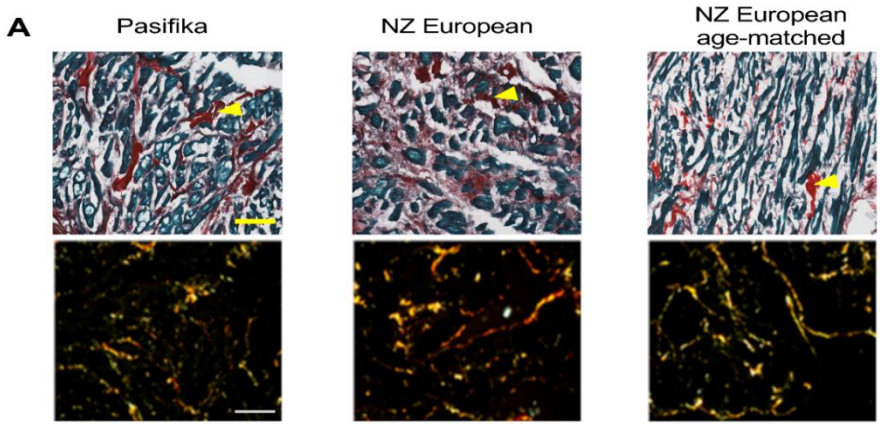


Figure 2B: Color-based quantification of percentage area of RAA of CABG patients occupied by collagen (red). Collagen was significantly higher than 5% observed in healthy RAA (dotted line, from literature), but not different between ethnic groups. Data in bar graph is shown as mean ± SD, with individual patient data illustrated (n = 7) per group. Statistical differences from non-cardiac disease values were determined by one-sample Wilcoxon test versus control values obtained from the literature (# $p < 0.05$). Differences between ethnicities was determined by one-way ANOVA, with statistical significance set at $p < 0.05$, with Tukey's post-tests between all three groups conducted if appropriate. NZE = New Zealand European, NZE-AM = New Zealand European Age-Matched.

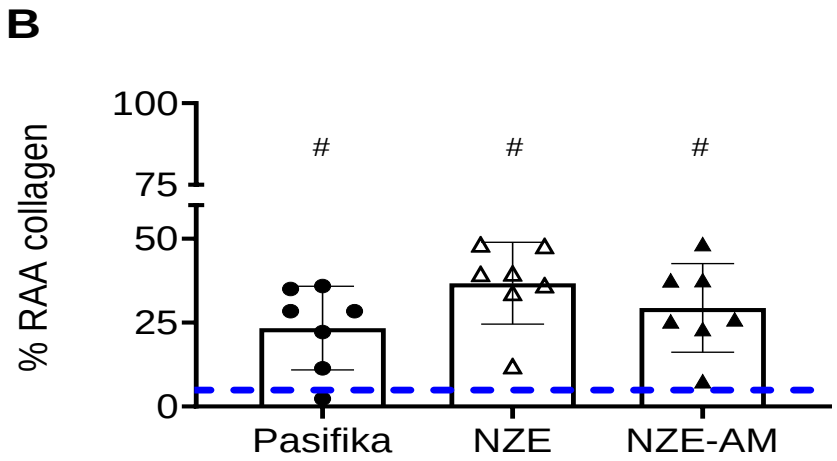


Figure 2C: Ratio of collagen I/III from color-based quantification. The ratio of collagen was not different from healthy value (dotted line) and no differences were observed between the groups. Data in bar graph is shown as mean ± SD, with individual patient data illustrated (n = 7) per group. Statistical differences from non-cardiac disease values were determined by one-sample Wilcoxon test versus control values obtained from the literature (# $p < 0.05$). Differences between ethnicities was determined by one-way ANOVA, with statistical significance set at $p < 0.05$, with Tukey's post-tests between all three groups conducted if appropriate. NZE = New Zealand European, NZE-AM = New Zealand European Age-Matched

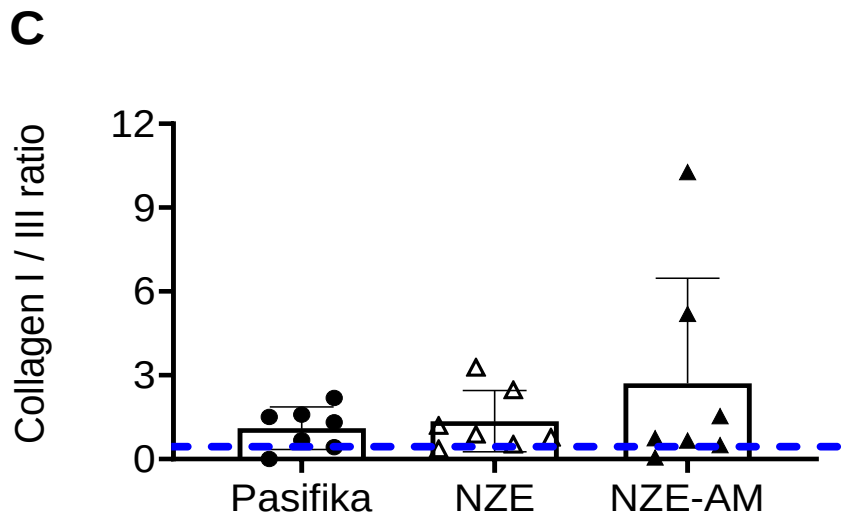


Figure 3: Myocardial fat and cardiomyocyte composition of the right atrial appendages (RAA) of coronary artery bypass graft (CABG) patients.
Fig 3A: Representative RAA images from each ethnic cohort stained with Oil-Red-O (ORO; red stain). Scale bar represent 10µm. The lipid deposits were mainly within individual cardiomyocytes (white arrows), with occasional extracellular fat depositions noted (yellow arrow head).

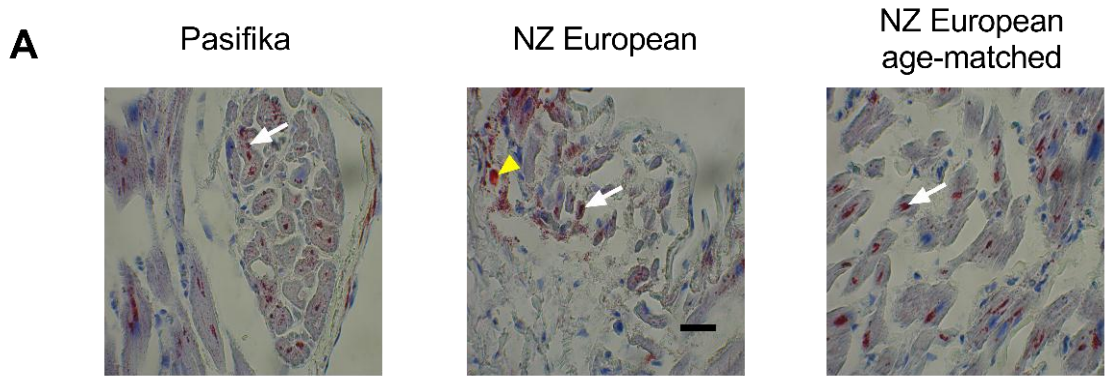


Figure 3B: Color-based quantification of percentage area of RAA of CABG patients occupied by lipid (red). Percentage fat was higher than 1% observed in healthy RAA (dotted line, from literature), but not different between ethnic groups. Data in bar graphs is shown as mean ± SD, with individual patient data illustrated (n = 7) per group. Statistical differences versus healthy atrial tissue values were determined by one-sample Wilcoxon test versus literature obtained hypothetical values (# $p < 0.05$). Differences between ethnicities was determined by one-way ANOVA, with statistical significance set at $p < 0.05$, with Tukey's post-tests between all three groups conducted if appropriate. NZE = New Zealand European, NZE-AM = New Zealand European Age-Matched.

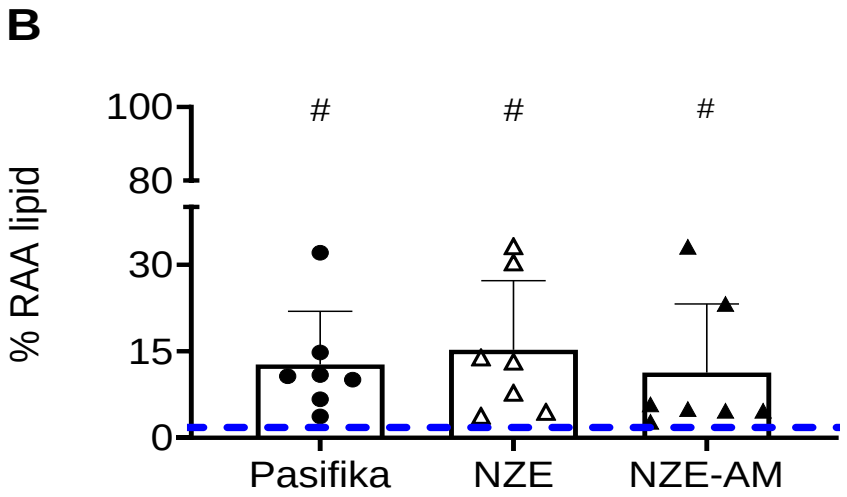


Figure 3C: Color-based quantification of percentage area of RAA of CABG patients occupied by cardiomyocytes (blue). The area occupied by myocytes was significantly lower than 65% observed in healthy RAA (dotted line, from literature). Data in bar graphs is shown as mean ± SD, with individual patient data illustrated (n = 7) per group. Statistical differences versus healthy atrial tissue values were determined by one-sample Wilcoxon test versus literature obtained hypothetical values (# $p < 0.05$). Differences between ethnicities was determined by one-way ANOVA, with statistical significance set at $p < 0.05$, with Tukey's post-tests between all three groups conducted if appropriate. NZE = New Zealand European, NZE-AM = New Zealand European Age-Matched.

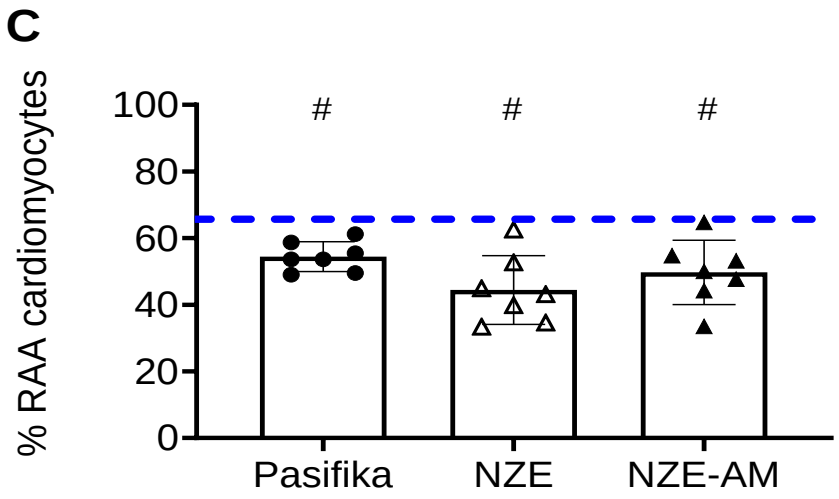


Figure 3D: Overall contribution of collagen, lipid, cardiomyocytes and other structures to the composition of the RAA, expressed as a percentage of whole area. Data in pie charts is shown as mean percentage composition.

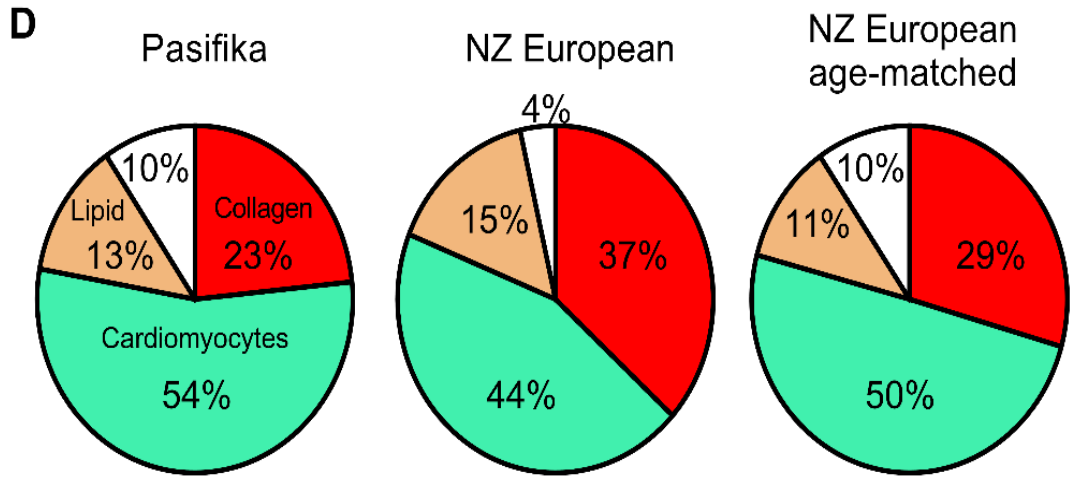


Figure 4: Association of percentage lipid in the RAA to A) systolic, B) diastolic and C) mean arterial blood pressure (mmHg) in Pasifika (closed circles), NZ European (open triangles) and NZ European age-matched (closed triangles) for n = 7 per group. Significant positive correlations (non-parametric Spearman correlation r) of percentage lipid within RAA to systolic (r = 0.94, p = 0.017), diastolic (r = 0.87, p = 0.033) and mean arterial blood pressure (r = 0.89, p = 0.033) were detected in the Pasifika cohort (lines), but not the NZ European cohorts (r < 0.64, p > 0.05).

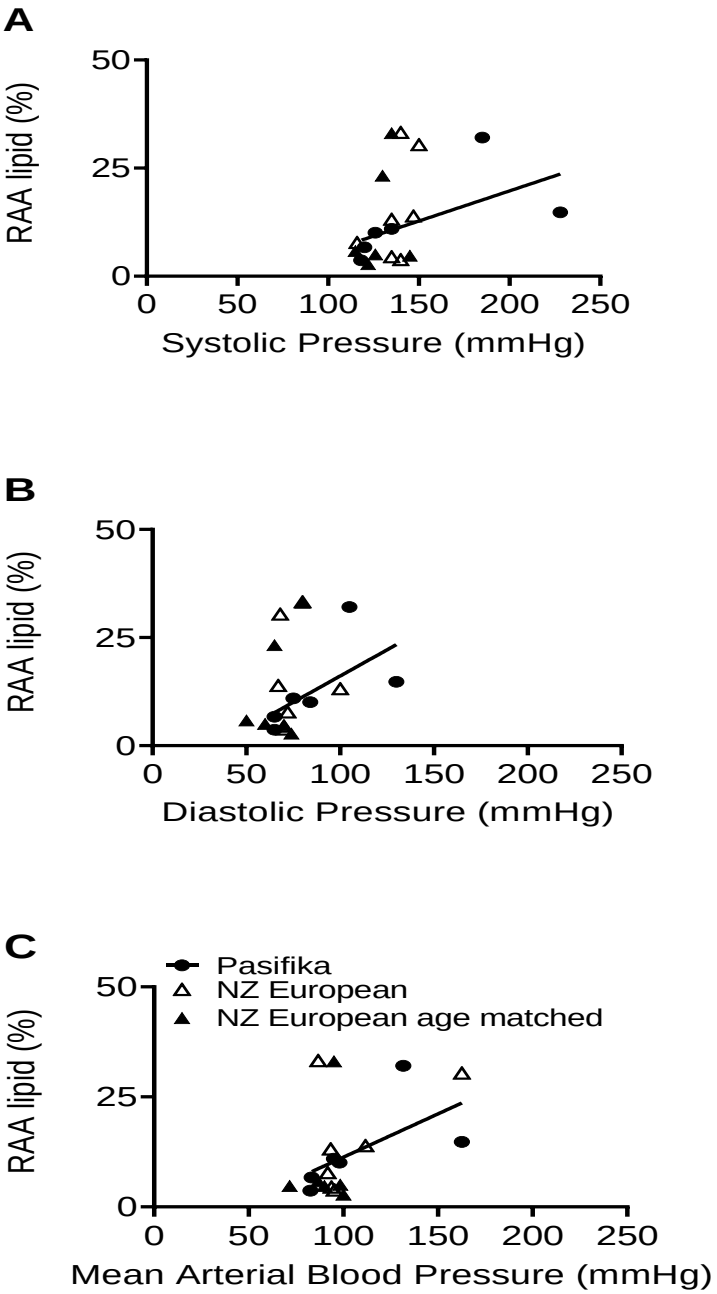
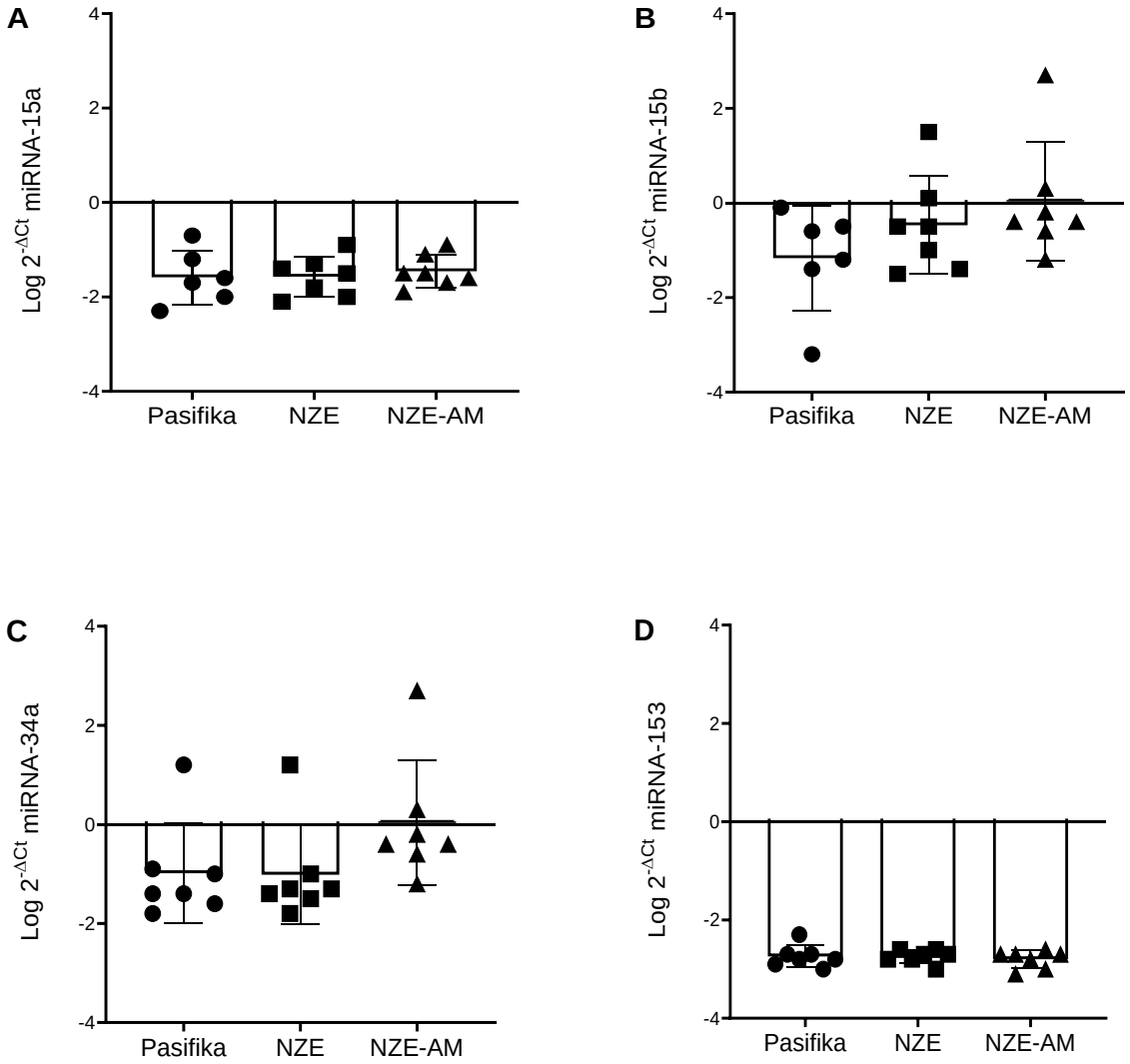


Figure 5: MicroRNA expression in RAA of CABG patients. Expression of miRNA-15a, 15-b, -34a and -153 in right atrial appendage (RAA) tissue of coronary artery bypass graft (CABG) patients are not altered by ethnicity. **A) miRNA-15a**, **B) miRNA-15b C) miRNA-34a and D) miRNA-153**. Data is expressed as Log 2-ΔCt of miRNA compared to endogenous control (average of miRNA-24 and -16), with mean ± SD, n = 7 per group with individual patient samples shown. One-way ANOVA detected no significant differences between ethnicities. NZE = New Zealand European, NZE-AM = New Zealand European Age-Matched.



DISCUSSION:

The main finding of this study was that no ethnic differences were found in histopathological assessment of right atrial tissue or in the expression of four miRNAs whose targets are associated with fibrosis and cardiomyocyte loss in a small population of Pasifika and New Zealand European cardiac surgery patients.

Pasifika and New Zealand Māori people tend to have poorer health outcomes compared to Pākehā population [5]. Differences in socio-economic and classical CVD risk factors are known contributing factors to the health inequality [12]. However, these factors do not fully explain the ethnic inequality and it is likely that other factors are also in play. Ethnic differences in CVD risk [7, 35], even following adjustment for other risk factors, have been detected [36] alongside ethnic differences in the heart structure and function [37]. This suggests that underlying physiological factors could play a role, as well. Our study focused on determining whether the composition of the heart tissue was different between Pasifika and NZ Europeans in Aotearoa undergoing surgical interventions for coronary artery disease. We found no differences in tissue composition between the ethnic backgrounds of CABG patients that could explain the health inequality in the Pasifika population. This suggests that, at least when CABG is clinically indicated, there seem to be no underlying differences in cardiac composition quantified in this study between the ethnic cohorts. Whether differences exist in the healthy population, people with preclinical disease or within the female population remain undetermined.

Hallmarks of a dysfunctional myocardium are marked ECM deposition, epicardial and intra-myocardial fat deposition, and loss of ventricular myocytes [38]. These histopathological changes were all detected within the RAA (compared to established values in the literature). A fibrotic response to the presence of CVD was detected across all ethnicities of the cardiac surgery patients in this study, with at least doubling of the cardiac collagen content compared to values obtained in tissue from healthy controls [32]. The extent of fibrosis observed in our patients was very similar to the elevated levels observed in American patients undergoing CABG [39]. The small group size limits meaningful statistical comparison within the different pacific ethnicities, but there was no clear difference between fibrosis in patients that identified as Samoan or Cook Island Māori. Therefore, it appears that the fibrotic response of the right atrium to coronary artery disease is similar independent of ethnic background, both in NZ and across the globe.

Fibrotic deposition in the myocardium of coronary artery disease patients has been shown to be located both perivascular and interstitial [17]. In our RAA samples there was minimal vascular tissue present hence the increase in fibrosis is assumed to be primarily

interstitial. In general, the diffuse spatial distribution of the fibrosis, alongside minimal change in RA dimensions and the relatively small reduction in cardiomyocytes within the RAA, was more indicative of reactive fibrosis to chronic stressors, rather than replacement scar tissue [40].

The two major collagen types within the extracellular matrix of the myocardium are generally type I and type III, and alterations to the ratio is one way that changes to the ECM can adversely affect cardiac functioning [41]. Previous studies have shown that increases in collagen I/III ratio are detected in end-stage dilated cardiomyopathy patients with reduced EF (<50%) [42]. Whilst we observed no significant change in the ratio in CABG patients, we did note a large variation in collagen I/III ratio in the age-matched NZ European group, with two individuals demonstrating a larger ratio that the other patients (Figure 2C). The clinical relevance of this is uncertain. Future studies including patients with EF < 50% would be required to see if the increase in collagen I/III ratio is present in CABG patients.

Intra-myocardial fat, the deposition of lipid droplets within cardiomyocytes, was the main location of lipid deposition within the RAA (Figure 3A), with only occasional extracellular fat deposits. Despite the knowledge that intra-myocardial fat is known to increase with age, obesity and metabolic syndromes [32, 43], we did not detect any association of fat deposition to blood glucose, possibly as a consequence of our matching of groups for these factors.

All the hallmarks of a dysfunctional myocardium were all detected within the RAA of CABG patients, with no ethnic differences observed. It is plausible that the stress on the myocardium from the presence of severe atherosclerotic disease is a much stronger driver of fibrosis, than ethnicity. This theory is supported by an association of diffuse interstitial fibrosis and prior cardiovascular events [44], alongside less fibrosis in patients with cardiac valve, not coronary artery, disease [45].

All patients in our study had BMI> 30 kg/m2, which is known to have a direct influence on fibrosis [46] and fat [42] deposition. We noted some ethnic variations in the relationship between blood pressure and fatty infiltration, with a strong association in Pasifika, but not other cohorts. These differences in correlations whilst, subtle, were identified despite our study design to effectively match patients in all groups to a wide range of clinical and cardiovascular parameters. Recently we indicated that BMI was associated with epicardial adipose tissue thickness in NZ European patients, but not in Pasifika/Māori patients [47]. Combined with the current correlation data from this study, further research is needed into the association between mechanisms and consequences of fat

deposition in Pasifika and New Zealand European peoples.

Greater up-regulation of the age and cardiac fibrosis related miRNA-34a [18] could be responsible for the earlier manifestation of clinically indicated cardiac disease in Pasifika, but this was unsupported by our data. However, with a focus just on the NZ European cohort, an age-related increase in miRNA-34a was apparent (Figure 5C). Hence, further investigation is warranted as our low group sizes may have masked potential ethnic differences. Previously, in RAA samples from the HeartOtago tissue collection, a down-regulation of miRNA-15a/b was found in diabetic patients with coronary artery disease of NZ European origin, compared to patients without coronary artery disease which was shown to influence cardiac fibrosis [17]. Given that within each of our ethnic cohorts no differences in miRNA-15a or -15b was observed between patients with or without diabetes, it is most likely that a similar overall down-regulation of miRNA-15a or 15b has occurred in our CABG patients, independent of ethnicity.

Our study has several limitations, with the most important one the relatively small number of patients included. To minimize imbalances between the ethnic groups, the number of tissues examined were matched to the available tissue of our heterogenous Pacific Islanders cohort (n = 7). This low number is most likely caused by the relative low number of Pasifika people living in the geographical area of Otago and Southland that present for cardiac surgery at the Dunedin Hospital (1%) compared to national rates. However, problems of Pasifika with access to health care cannot be excluded. The small group size also prevented any meaningful comparisons between the Pasifika sub-groups, where differences have recently been highlighted in CVD prevalence [48]. Because gender differences of cardiac fibrosis have been identified in mice [49] and humans [33], it would be interesting to explore in the future the cardiac histopathological status of female Pasifika, as well. Care should also be taken in interpolating our findings in the RAA directly to the whole heart. Indeed, we have previously shown chamber specific differences in Ca^{2+} handling proteins in HeartOtago patient samples [50], whereas others showed chamber-specific variation in fibrosis [51, 52] and fat [32]. However, the right atrium is important for right ventricular function and especially for electrical conduction with approximately 30% of all cardiac arrythmias originating from the right atrium [53].

CONCLUSION:

Our study suggests that despite the greater risk of premature death and earlier requirements for surgical intervention for CVD of the Pasifika people from Aotearoa, this is not explained by differences in histopathology of the heart. Future investigations should investigate other potential physiological reasons, in parallel to

the socioeconomic reasons, why Pasifika reach a clinical indication for cardiac surgery years before NZ Europeans.

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