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Differences in visceral adipose tissue and biochemical cardiometabolic risk markers in elite rugby union athletes of Caucasian and Polynesian descent

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Abstract

Polynesian individuals are leaner with greater musculature than Caucasians of an equivalent size, and this genetically different morphology provides a physique that is often compatible with success in a number of sports, including rugby union. Evidence indicates that Polynesians have greater stores of absolute and relative abdominal fat mass and this is known to confer cardiometabolic risk. The aims of this study were to 1) explore the relationship between ethnicity, visceral adipose tissue (VAT), and cardiometabolic disease risk markers in elite Caucasian and Polynesian rugby union athletes, and 2) assess the impact of a pre-season training program on these markers. Twenty-two professional rugby union athletes of Caucasian (n=11) and Polynesian (n=11) descent underwent physique assessment via surface anthropometry, dual-energy X-ray absorptiometry, and magnetic resonance imaging before and after an 11-week pre-season. A fasted blood test was undertaken at both time points. Compared to Caucasians, at baseline Polynesians displayed significantly higher VAT (771 ± 609 cm^3 vs 424 ± 235 cm^3; p=0.043), triglycerides (1.0 ± 0.9 mmol/L vs 0.6 ± 0.2 mmol/L; p=0.050), and low-density lipoprotein cholesterol (3.1 ± 0.9 mmol/L vs 2.3 ± 0.7 mmol/L; p=0.019). Similar changes were observed in both groups over the pre-season period in VAT and blood biochemical markers. Polynesian rugby union

athletes were more likely than Caucasians to exhibit risk factors associated with cardiometabolic disease, such as elevated VAT and unfavourable lipid profiles. Further longitudinal research is required to identify and explain the short- and long-term risk of cardiometabolic disease in athletes of Polynesian descent.

Key Words

VAT, cholesterol, MRI, DXA, body fat, abdominal fat.

Introduction

Rugby union is a contact team sport which places significant physiological demands on the athlete (Bradley et al., 2015). The development of lean mass is desirable to enhance speed, strength and power, which are fundamental attributes for competitive success (Olds, 2001). Additionally, body mass has been identified as being strongly associated with overall team performance (Olds 2001, Sedeaud et al., 2012). The emphasis on muscularity and overall size may explain the anecdotal rise in Polynesian athletes competing at the elite level in rugby union. Additionally, it may rationalise the relative success of national teams from Pacific nations in international competition, with Fiji, a country with a population of 900,000 having a comparable world ranking to that of France with a population of 67 million (as of May 2019). Polynesians have been shown to be significantly leaner with greater muscle mass than Caucasians at an equivalent body mass index (BMI) (Swinburn, Ley, Carmichael, & Plank, 1999). Furthermore, they display proportionally higher levels of fat free mass and lower levels of fat mass after adjustments for stature and mass (Rush, Freitas, &

Plank, 2009). As such, the genetic morphology of Polynesians appears to predispose them to a physique compatible with success in rugby union.

A large proportion of elite rugby union athletes are defined as overweight or obese using the traditional Caucasian ethnicity BMI cut-offs of 25–30 kg/m^2 and >30 kg/m^2, respectively (Zemski, Keating, Broad, Marsh, & Slater, 2018), but BMI is not suitable for estimating fat mass in athletic populations (Ackland et al., 2012). Waist circumference (WC) (Alberti, Zimmet, & Shaw, 2006) and waist to height ratio (WHt) (Swainson, Batterham, Tsakirides, Rutherford, & Hind, 2017) are commonly used to identify increased disease risk associated with higher abdominal adiposity, with a recent study identifying WHt to be a superior measure for obesity characterisation in adults (Swainson et al., 2017). However, the application of these measures to an athletic population with unique morphology, such as that found in rugby union, is questionable due to the different ratio of fat and lean mass in athletic individuals compared to the general population (Ackland et al., 2012). It is now recognised that the topography of body fat is a better predictor of cardiometabolic complications than the overall amount of fat mass (Tchernof & Despres, 2013). Indeed, high levels of visceral adipose tissue (VAT), which encompasses fat stores in the intra-abdominopelvic region bounded by the abdominal wall and pelvic floor (Shen et al., 2003), is an established marker for cardiometabolic risk and is independent of total body mass, total body fat and subcutaneous adipose tissue (SAT) (Despres & Lemieux, 2006). Additionally, VAT has been identified as an important risk factor for atherosclerosis in men (Lear et al., 2007) and there is evidence identifying a relationship between VAT and cardiovascular endpoints (Hughes-Austin, Larsen, & Allison, 2013). This is of particular pertinence to Polynesians, who

possess greater stores of abdominal fat mass than Caucasians in both absolute and relative terms (Rush et al., 2009). Despite Polynesians having some of the highest rates of obesity and cardiometabolic disease worldwide (Ng et al., 2013), VAT has not been reported in general Polynesian populations.

Studies in overweight and obese populations have revealed that exercise lowers risk of cardiometabolic disease risk (Barry et al., 2014), reduces VAT (Ismail, Keating, Baker, & Johnson, 2012), and lessens the adverse effects of obesity on morbidity and mortality (Fogelholm, 2010). Nonetheless, "supersized" athletes – those who purposefully maximise their lean and/or fat mass to optimise performance is a particular sport and/or position – have been shown to display signs of elevated cardiometabolic disease risk, including compromised lipid profiles (Guo, Zhang, Wang, Guo, & Xie, 2013; Tucker et al., 2009) and higher VAT levels (Bosch et al., 2014; Murata, Oshima, Torii, Taguchi, & Higuchi, 2016) compared to non-heavyweight athletes or non-athletic controls. It is possible that elevated lipid markers in the presence of regular training may be an indicator of cardiovascular disease risk. Indeed, American football (NFL) linemen, the largest athletes in the sport, have almost double the prevalence of metabolic syndrome post-retirement compared to non-linemen (59.8 % vs 30.1 %; $p < 0.001$) (Miller et al., 2008). Recently, elite rugby union athletes of Polynesian decent were shown not to have significantly different VAT compared to Caucasians (Zemski et al., 2018). However, this observation was based on single slice MRI and there is no general agreement as to the best location to take this measurement (Schweitzer et al., 2015). Further, studies sampling single slice VAT have been observed to be influenced by ethnicity (Demerath et al., 2007). No study has investigated levels of VAT using a volumetric measure in elite athletic

populations, nor have ethnic differences in biochemical markers of cardiometabolic disease risk been examined in an elite rugby union population.

Given the increased number of Polynesians in professional rugby union, and the increasing size of elite athletes within the sport, an understanding of cardiometabolic disease risk in this population would be valuable to practitioners to assist with athlete management from a health care perspective. Therefore, the aims of this study were to: 1) explore the relationship between ethnicity, VAT, and cardiometabolic disease risk markers in elite rugby union athletes of Caucasian and Polynesian descent; and 2) assess the impact of a pre-season training program on levels of VAT and biochemical markers of cardiometabolic disease risk.

Materials and Methods

Participants

Twenty-two male professional rugby union athletes were recruited via their involvement in a Super Rugby franchise. Informed consent was obtained from all athletes included in the study, and protocols were submitted to, and approved by, the relevant institutional review boards and ethics committees for testing of human subjects.

At the time of consent the athletes provided the ethnicity of their grandparents via open ended questions. As this study investigated the role of phenotype expression, grandparental heritage was chosen as in previous research (Zemski et al., 2018). The

126 athletes were ascribed a specific ethnicity if three or four of their grandparents were of
127 the same ethnicity.
128
129 ***Study design***
130
131 As part of the physical preparation for the Super Rugby season, the athletes undertook
132 a high-volume, high-intensity, 11-week pre-season program (November – February)
133 with the aims of increasing strength and power and improving aerobic and anaerobic
134 fitness; some of which can be favourably impacted by strategic body composition
135 manipulation (Bilsborough, Greenway, Livingstone, Cordy, & Coutts, 2016). During
136 the first three days of pre-season, the athletes undertook routine physique assessment
137 via surface anthropometry and dual-energy X-ray absorptiometry (DXA). In addition,
138 they received a magnetic resonance imaging (MRI) scan of their abdominal cavity.
139 Athletes were re-assessed using the same techniques within the final three days of the
140 pre-season period. A fasted blood test was undertaken at the same time points.
141
142 ***Surface anthropometry***
143
144 An International Society for the Advancement of Kinanthropometry (ISAK) Level 3
145 accredited anthropometrist with a historical technical error of measurement (TEM) of
146 1.7% for sum of seven skinfolds performed all measurements. Body mass was
147 assessed using electronic scales (A&D Mercury, Adelaide, Australia) to 0.1 kg
148 accuracy, and stature measured using a mobile stadiometer (Seca 213, Birmingham,
149 UK) to 0.1 cm accuracy. Both measurements were made on arrival at the testing
150 facility after an overnight fast and with bladder voided. Athletes were provided with

guidelines on what foods and fluids to consume the day before testing, including the time at which they were to consume their last meal. This was replicated at both testing time points. Skinfolds were assessed using Harpenden calipers (British Indicators, Hertfordshire, UK) to 0.1 mm precision. All anthropometric equipment was calibrated as recommended by the manufacturers.

Skinfold and waist circumference measurements were made using ISAK techniques (Norton et al., 2006). Skinfolds were assessed across seven sites: triceps, subscapular, biceps, supraspinale, abdominal, mid-thigh, and medial calf. For athletes for whom a reliable skinfold could not be taken at the abdominal site 5 cm from the umbilicus, the site was moved to 10 cm from the umbilicus at both time points. Waist circumference was measured at the level of the narrowest point between the lower costal border and the iliac crest and taken at the end of normal expiration. All measurements were undertaken in duplicate to establish within-day retest reliability. If the difference between the duplicate measures exceeded 4% for an individual skinfold or 1% for waist circumference, a third measurement was taken. The mean of duplicate or the median of triplicate anthropometric measurements were used for all subsequent analysis. BMI was calculated using the formula mass (kg) divided by stature (m) squared. WHt ratio was calculated using the formula waist circumference (cm) divided by stature (cm).

Dual-energy X-ray absorptiometry

All athletes received whole body composition analysis via scans on a fan-beam DXA system (Hologic Discovery A, Hologic, Bedford, MA), with Apex 13.4.2:3 software

(Hologic, Bedford, MA). The scanner was calibrated daily using a phantom as per manufacturer guidelines for quality control purposes.

Scanning protocols were implemented using proven techniques to maximise technical reliability and minimise error (Nana, Slater, Stewart, & Burke, 2015). Specifically, the athletes were scanned prior to food and fluid ingestion, or exercise, early in the morning (5:00 – 8:30 am). Athletes were provided with guidelines on what foods and fluids to consume the day before testing, including the time at which they were to consume their last meal. This was replicated at both testing time points. The athletes were scanned wearing sports shorts and those taller than the defined 196 cm scanning boundary were subject to two scans. The first scan was used to capture the body from the menton (the inferior point of the mandible) down whilst the head was positioned in the Frankfort plane. After body repositioning on the scanner and realignment of the head into the Frankfort plane, a second scan was taken to capture from the menton up to the vertex of the head. The results were then combined post-analysis to produce whole body composition scans. For positioning consistency the same experienced and qualified technician performed all measurements using the Nana et al. positioning protocol previously described (Nana et al., 2015). The same qualified technician undertook all post-scan analysis, including the manual adjustment of all regions of interest. Auto positioning of the VAT area was used, with manual adjustments made to the edge of subcutaneous fat placement and visceral cavity area if required.

Magnetic resonance imaging

Abdominal SAT and VAT were measured on a PRISMA 3T MRI (Siemens Healthineers, Erlangen, Germany) at the Herston Imaging Research Facility (Brisbane, Queensland, Australia) by a qualified and experienced technician. A 32-channel spine array coupled with a 30-channel body array was utilised to perform the examination. Coverage extended from the diaphragm to the L5/S1 junction. The athletes were positioned either head first or feet first depending on their body habitus, and this position was repeated at the follow-up scan. Axial T1 weighted Dixon images were acquired in a single breath hold (TR 3.97 ms, TE 1.23/2.46 ms, flip angle 9°, matrix 320X240) with slice thickness of 4mm and no inter-slice gaps. The field of view (FOV) was 450 mm in order to include the skin surface.

Cross-sectional areas and volumes of both abdominal SAT and VAT from L5/S1 to the diaphragm were measured by semi-automated specialized software (Slice-O-matic version 5.0; Tomovision, Montréal, Canada). SAT was quantified using the "mathematical morphology" function and VAT using the "region growing" function, with thresholds adjusted manually for each slice. All images were analysed by a single trained observer who was not informed of athlete ethnicity and playing position. Examples of the output provided by image analysis process is shown in Appendix 1. Presently, there are no established reference ranges indicating increased risk or cardiometabolic complications for VAT volume given it is a relatively new assessment. Intra-observer variability was assessed by re-analysing 11 randomly selected scans after a minimum 3-month interval.

Blood biochemistry

Venous blood (20ml) was collected from the antecubital vein after an overnight fast (>10 hours). On the same day serum glucose, insulin, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) concentrations were analysed by a commercially accredited laboratory (QML Pathology, Specialist Diagnostic Services Pty Ltd, NSW, Australia). Analysis was conducted using a Siemens ADVIA 1800 Chemistry System (Siemens Healthineers, Erlangen, Germany) with the associated Siemens testing kit and recommended reagents. Blood was centrifuged for 10 minutes at 3000 g. This allowed the red blood cells to be collected at the bottom of the tube below the gel, and the serum to be collected at the top of the tube. The serum was analysed to test fasting glucose and insulin, and a full lipid profile, which included TG, TC, HDL-C and LDL-C.

Training

Athletes undertook an 11-week pre-season program. This followed a period of 4-weeks of leave, which included an active rest schedule of two strength and two conditioning sessions a week. The pre-season comprised a 4-week supervised training block prior to a 2-week unsupervised maintenance block, followed by another 5-week supervised training block. Throughout each training week, technical (x2/week) and tactical (x4/week) rugby sessions along with sessions to improve physical qualities and body composition were performed (speed/agility x1/week, strength x4/week, conditioning x3-4/week, boxing x1/week). Weekly training time was approximately 15 hours, with additional time spent on recovery and regeneration modalities (flexibility, mobility, massage, hydrotherapy and physiotherapy). All athletes were

injury and illness free at the start of the pre-season period, and did not suffer from any significant injuries or illnesses that meaningfully restricted their training during the study period. All athletes were under the management of an experienced sports dietitian and received individualised dietary plans and group education sessions aimed at supporting training adaptations throughout the pre-season period.

Statistical analysis

Statistical analysis procedures were completed using SPSS (Version 22.0, IBM Corp., Armonk, NY) and Microsoft Excel 2011 (Microsoft, Redmond, WA, USA). Before analysis, assumptions of normality in the data were made using the Shapiro-Wilk test and visualisations of normality histograms and Q-Q plots. If data were not normally distributed they were log transformed using the natural logarithm for all subsequent analyses. Independent t-tests were used to test for differences in body composition traits and cardiometabolic risk markers according to ethnicity at baseline. A one-way analysis of covariance (ANCOVA) was conducted to determine how changes in body composition over the pre-season period varied by ethnicity, with baseline measures entered as a covariate. Bonferroni post hoc corrections were applied. Correlations were calculated using Pearson's and Spearman's correlations for normally distributed and nonparametric data respectively, to assess the relationship between body composition and cardiometabolic risk variables at baseline, and for changes over the pre-season period. For correlations, coefficients were qualitatively ranked by magnitude, with the strength of correlation coefficients defined as trivial, $r < 0.1$; small, $0.1 \leq r < 0.3$; moderate, $0.3 \leq r < 0.5$; large, $0.5 \leq r < 0.7$; very large, $0.7 \leq r < 0.9$; almost perfect, $0.9 \leq r < 1.0$; and perfect, $r = 1.0$. Data are presented as mean ±

standard deviation (SD), or median (inter-quartile range; IQR) for non-parametric variables, with statistical significance for all analyses defined *as* $p \leq 0.05$. Intraclass correlation coefficients (ICC) were used to determine the test-retest reliability, whilst the coefficient of variation (CV) was calculated (standard deviation divided by the mean) to show the extent of absolute variability.

Individual changes in DXA measures were evaluated through the application of least significant change (LSC) derived from precision data from a group of resistance trained athletes on the Hologic Discovery A scanner (Zemski et al., 2019). Precision error was calculated as root-mean-square standard deviation (RMS–SD), with LSC subsequently derived as RMS–SD x 2.77 (95% confidence interval; 95%–CI) (Baim et al., 2008). LSC values were also created using the same methods for surface anthropometry measures and MRI analysis using data collected within this population.

Results

All twenty-two athletes (age 23 ± 3 years; stature 186.8 ± 8.4 cm; mass 101.5 ± 13.7; BMI 29.0 ± 2.5 kg/m^2) were able to be ascribed an ethnicity, with 11 identifying as Caucasian, and 11 as Polynesian. The ICC (95% confidence interval) for VAT was 1.00 (CI 0.99–1.00) and for SAT was 1.00 (CI 0.98–1.00) with a CV of 2.0% and 2.7%, respectively. The LSC- 95% CI value for the sum of 7 skinfolds was 0.7 mm (1.9% CV), 0.6 cm (0.5% CV) for waist circumference, 129.2 cm^3 (4.8% CV) for SAT volume, and 28.3 cm^3 (5.1% CV) for VAT volume.

300

301 Descriptive characteristics based on ethnicity are presented in Table 1. Differences
302 were found between ethnicities at baseline, with Polynesians having significantly
303 higher VAT (771 ± 609 cm^3 vs 424 ± 235 cm^3; $p = 0.043$), android fat mass
304 percentage (19.4 ± 5.0 % vs 14.5 ± 3.8 %; $p = 0.020$), TG (1.0 ± 0.9 mmol/L vs 0.6 ±
305 0.2 mmol/L; $p = 0.050$), LDL-C (3.1 ± 0.9 mmol/L vs 2.3 ± 0.7 mmol/L; $p = 0.019$)
306 and WHt (0.50 ± 0.03 vs 0.47 ± 0.02; $p = 0.019$), whilst trending towards higher SAT
307 (3424 ± 1529 cm^3 vs 2279 ± 1014 cm^3; $p = 0.068$) and TC (5.1 ± 0.9 mmol/L vs 4.4 ±
308 0.8 mmol/L; $p = 0.057$).

309

310 Significant correlations were recorded at the start of pre-season with both SAT and
311 VAT in relation to other measures of adiposity including skinfolds, WC, and absolute
312 and relative android and gynoid fat (Table 2). Large correlations were noted between
313 VAT ($r = 0.564$, $p < 0.01$) and SAT ($r = 0.435$, $p < 0.05$) with TC, and very large
314 correlations between VAT ($r = 0.709$, $p < 0.01$) and SAT ($r = 0.705$, $p < 0.01$) with
315 TG.

316

317 Ethnicity was found to be significantly related to changes over the pre-season period,
318 with Polynesians having greater reductions in WC (-2.8 ± 1.6 cm vs -0.7 ± 1.2 cm; F
319 $= 9.208$, $p = 0.007$) and WHt (-0.02 ± 0.009 vs -0.004 ± 0.006; $F = 7.206$, $p = 0.015$),
320 whilst Caucasians had greater reductions in TC (-0.13 ± 0.32 mmol/L vs -0.08 ± 0.68
321 mmol/L; $F = 5.543$, $p = 0.029$) (Table 1).

322

323 Applying the LSC model, individual changes are shown in Table 3. Twenty-one
324 (95%) athletes had reductions in VAT (exceeding LSC-95% CI) and skinfolds over

the pre-season period, whilst 19 (86%) decreased SAT and android fat mass percent (Figure 1). A similar proportion of Caucasians and Polynesians athletes made meaningful changes in all measures assessed, with the exception of WC in which a larger proportion of Polynesians (91%) made a significant decrease in comparison to Caucasians (45%).

Large correlations in changes over the pre-season were found between VAT and skinfolds ($r = 0.575$, $p < 0.01$), WC ($r = 0.578$, $p < 0.01$), total fat mass ($r = 0.496$, $p < 0.05$), android fat mass ($r = 0.462$, $p < 0.05$), and gynoid fat mass ($r = 0.491$, $p < 0.05$). Similar or slightly larger and stronger correlations were seen between SAT and skinfolds ($r = 0.689$, $p < 0.01$), WC ($r = 0.558$, $p < 0.01$), total fat mass ($r = 0.557$, $p < 0.01$), android fat mass ($r = 0.625$, $p < 0.01$), and gynoid fat mass ($r = 0.488$, $p < 0.05$).

Discussion

In this research we were the first to assess volumetric measures of VAT in an elite athlete population and to adopt an individualised approach to the analysis of pre-season adiposity changes in elite rugby union athletes. The main findings were that: (1) athletes of Polynesian descent had significantly different abdominal adiposity distribution and lipid profiles compared to Caucasian athletes; and (2) the majority of athletes achieved meaningful and favourable reductions in both abdominal VAT (95%) and SAT (86%) over the pre-season period.

Prior to this study, there was limited research on visceral adiposity in athletic populations. Bosch et al. (2014) determined that NFL linemen (mass 137.1 ± 11.7 kg; BMI 37.3 ± 3.5 kg/m^2; percent fat 27.0 ± 6.0 %) have high levels of VAT as estimated by DXA compared to non-linemen (1.2 ± 0.6 kg vs. 0.3 ± 0.2 kg). Similarly, heavyweight judo athletes possessed relatively higher VAT measured by single slice MRI at L4/L5 compared to non-heavyweight athletes (91 ± 39 cm^2 vs 33 ± 14 cm^2) (Murata et al., 2016), which would have placed many above the 100 cm^2 diagnostic threshold for increased cardiometabolic disease risk (Pickhardt, Jee, O'Connor, & del Rio, 2012). It has previously been identified that 37% of athletes in an elite rugby union population were above the threshold for increased risk via single slice MRI, whist no differences were found between ethnicities (Zemski et al., 2018). However, this was using reference ranges developed in older and more obese populations and, therefore, the application to well-trained athletes was uncertain. Furthermore, single slice measures may be affected by ethnicity (Demerath et al., 2007), and Polynesian individuals have greater absolute and relative body fat distribution in the abdominal region (Rush et al., 2009). In the current study, elite Polynesian rugby athletes had higher VAT, and trended towards having higher SAT than Caucasians, despite no statistically significant difference in total fat mass or relative fat mass. It is important to note that one Polynesian athlete had a considerably higher VAT measurement than the other ten (Figure 1). As this was a statistical but not clinical outlier, we retained this in the primary analysis. Removal of this athlete from the group mean analysis weakened the ethnicity difference in VAT marginally (from $p = 0.043$ to $p = 0.070$). Moreover, the VAT results for all athletes were investigated after being adjusted for stature, but this had no effect on the outcomes,

indicating the relative size of the individual did not make a difference to VAT accumulation.

"Supersized" professional strength sport athletes in unlimited body weight categories (Guo et al., 2013) and NFL linemen (Tucker et al., 2009) have exhibited elevated blood lipid profiles compared to smaller stature athletes and/or non-athlete controls. Further, NFL linemen have an increased incidence of metabolic syndrome compared to non-linemen post-career (59.8 % vs 30.1 %; $p < 0.001$) (Miller et al., 2008), and a higher BMI and/or WC has been associated with subclinical atherosclerosis and cardiometabolic risk in retired NFL athletes (Trexler et al., 2018). However, comparisons based on ethnicity in elite athlete populations have not previously been made. Although in this study the group mean all variables were considered to be within the normal ranges, likely owing to the young age and high activity levels of the cohort, Polynesians had higher TG and LDL-C, and a trend towards higher TC, relative to Caucasians. A number of Polynesian athletes had blood lipids that were outside of the low risk targets (Tonkin et al., 2005), including two for TC (> 6.0 mmol/L), one for TG (> 1.5 mmol/L), one for HDL-C (< 1.0 mmol/L), and six for LDL-C (> 3.0 mmol/L). Only one Caucasian had elevated LDL-C levels. No athletes were outside the normal range for fasted insulin or glucose measures.

Published data implies that Polynesians have some of the highest rates of obesity and cardiometabolic disease risk worldwide (Ng et al., 2014). It is noteworthy that, in the athletic population we studied in which all athletes were undertaking comparable training programs, elevated lipid profiles were still recorded in Polynesians. The higher TC and LDL-C concentrations in Polynesian athletes may place them at

elevated risk of post-career cardiometabolic complications when activity levels are prone to decline, as has been shown in other sports with "supersized" athletes (Miller et al., 2008; Trexler et al., 2018). This warrants further investigation of rugby union athletes after retirement, particularly given the greater focus on larger athletes is a relatively new phenomenon in the sport.

Elevated VAT has been associated with dysfunctional glucose and lipid metabolism (Despres & Lemieux, 2006). In our study, higher levels of VAT showed significant correlations with TC and TG levels. It has been suggested that increased physical activity acts as a protective barrier against cardiometabolic disease risk in the general population (Barry et al., 2014) as well as "supersized" athletes (Murata et al., 2016). Indeed, a recent meta-analysis in normal-weight and overweight/obese individuals reported high-intensity interval training (HIIT) has been shown to reduce VAT (Maillard, Pereira, & Boisseau, 2018). Specifically in athletic groups, elite sumo wrestlers have significantly lower visceral adiposity compared to obese controls, normal glucose and TG levels, and lower TC compared with non-obese controls (Matsuzawa, Shimomura, Nakamura, Keno, & Tokunaga, 1993). Furthermore, the significant reduction in VAT amongst Caucasians and Polynesians in this study at both the group and individual level, coupled with the absence of significant biochemical changes in cardiometabolic risk profiles, indicate that physical activity may be protective of adverse blood biochemical changes in elite athletes during their playing career. However, given "supersized" athletes have been reported to have a higher incidence of metabolic syndrome (Miller et al., 2008) and other obesity related complications post-career (Trexler et al., 2018), health status should continue to be monitored after retirement in conjunction with athlete follow-up and support.

The results of this study indicate that Polynesian rugby union athletes have higher levels of VAT and blood lipid markers than their Caucasian peers, with the underlying reasons for this possibly dating back to physiological traits that have evolved over millennia (Bindon & Baker., 1997). Future investigations encompassing longitudinal studies, incorporating end of season measures of VAT and blood biochemical markers to assess changes during the off-season period, would be valuable. Additionally, studies exploring cardiometabolic health and broader health issues of rugby union athletes post-retirement would be beneficial. This would add a holistic view to the research being undertaken on long-term health status of retired rugby union athletes (Hume et al., 2017).

There were some limitations identified with this study. First, it was not possible to accurately quantify dietary intake over a long period (Magkos & Yannakoulia, 2003), or the training associated energy expenditure of the athletes given the high-intensity nature of the training being undertaken (Drenowatz & Eisenmann, 2011) together with the frequent physical collisions (Bradley et al., 2015). This information may have afforded additional insight into the underlying reasons for changes and differences based on ethnicity in abdominal adiposity and blood biochemistry. In particular, it may have provided a reason for the increased VAT in a single Polynesian athlete, which was unexpected given the high training load and results of the other athletes. Secondly, information on the medical history of the athletes and their families would have allowed exploration into possible hereditary influences. This may have provided some insight into the underlying reason one athlete displayed significantly higher VAT. Of note is that the changes this athlete made to his

abdominal adiposity over the pre-season were in line with previous research, namely that VAT (1421 g) was lost preferentially to SAT (631 g) (Verheggen et al., 2016). Finally, the research was limited by the relatively small sample size, as is inherent in all elite-level studies due to the rarity and availability of elite athletes. Given this, the findings may not be representative of an entire ethnic group within a specific sport, or within a general athletic population. However, given the strength of the findings in this study, and the health data available in general Polynesian populations, while inter-individual variability in VAT is evident, it is likely ethnicity plays a role in cardiometabolic disease risk markers in "supersized" athletes.

This study identified significant differences in cardiometabolic disease risk factors based on ethnicity in elite rugby union athletes, with Polynesians having higher values for VAT and several lipid markers. Although athletes of both ethnicities had meaningful reductions to VAT as a result of pre-season training, it is possible that Polynesian athletes may be predisposed to the higher VAT and blood biochemistry markers associated with cardiometabolic disease risk. Further investigations are advocated to explore the underlying reasons for these findings, and the long-term cardiometabolic health implications in elite "supersized" Polynesian athletes.

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Declarations of Interest

All authors declare they have no conflicts of interest relevant to the context of this study.

References

Ackland, T. R., Lohman, T. G., Sundgot-Borgen, J., Maughan, R. J., Meyer, N. L., Stewart, A. D., & Muller, W. (2012). Current status of body composition assessment in sport: review and position statement on behalf of the ad hoc research working group on body composition health and performance, under the auspices of the I.O.C. Medical Commission. *Sports Medicine, 42*(3), 227-249.

Alberti, K. G., Zimmet, P., & Shaw, J. (2006). Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabetic Medicine, 23*(5), 469-480.

Baim, S., Binkley, N., Bilezikian, J. P., Kendler, D. L., Hans, D. B., Lewiecki, E. M. & Silverman, S. (2008). Official Positions of the International Society for Clinical Densitometry and executive summary of the 2007 ISCD Position Development Conference. *Jounral of Clinical Densitometry, 11*(1), 75-91.

Barry, V. W., Baruth, M., Beets, M. W., Durstine, J. L., Liu, J., & Blair, S. N. (2014). Fitness vs. fatness on all-cause mortality: a meta-analysis. *Progress in Cardiovascular Diseases, 56*(4), 382-390.

Bilsborough, J. C., Greenway, K., Livingstone, S., Cordy, J., & Coutts, A. J. (2016). Changes in anthropometry, upper-body strength, and nutrient intake in professional

Australian football players during a season. *International Journal of Sports Physiology and Performance, 11,* 290-300.

Bindon, J. R., & Baker, P. T. (1997). Bergmann's rule and the thrifty genotype. *American Journal of Physical Anthropology, 104*(2), 201-210.

Bosch, T., Burruss, T., Weir, N., Fielding, K., Engel, B., Weston, T., & Dengel. D. R. (2014). Abdominal body composition differences in NFL football players. *Journal of Strength and Conditioning Research, 28*(12), 3313-3319.

Bradley, W. J., Cavanagh, B. P., Douglas, W., Donovan, T. F., Morton, J. P., & Close, G. L. (2015). Quantification of training load, energy intake, and physiological adaptations during a rugby preseason: a case study from an elite European rugby union squad. *Journal of Strength and Conditioning Research, 29*(2), 534-544.

Demerath, E. W., Sun, S. S., Rogers, N., Lee, M., Reed, D., Choh, A. C., … Towne, B. (2007). Anatomical patterning of visceral adipose tissue: race, sex, and age variation. *Obesity (Silver Spring), 15*(12), 2984-2993.

Despres, J. P., & Lemieux, I. (2006). Abdominal obesity and metabolic syndrome. *Nature, 444*(7121), 881-887.

Drenowatz, C., & Eisenmann, J. C. (2011). Validation of the SenseWear Armband at high intensity exercise. *European Journal of Applied Physiology, 111*(5), 883-887.

Fogelholm, M. (2010). Physical activity, fitness and fatness: relations to mortality, morbidity and disease risk factors. A systematic review. *Obesity Reviews, 11*(3), 202-221.

Guo, J., Zhang, X., Wang, L., Guo, Y., & Xie, M. (2013). Prevalence of metabolic syndrome and its components among Chinese professional athletes of strength sports with different body weight categories. *PLoS One*, *8*(11), e79758.

Hughes-Austin, J. M., Larsen, B. A., & Allison, M. A. (2013). Visceral adipose tissue and cardiovascular disease risk. *Current Cardiovascular Risk Reports, 7,* 95-101.

Hume, P. A., Theadom, A., Lewis, G. N., Quarrie, K. L., Brown, S. R., Hill, R. & Marshall, S. W. (2017). A Comparison of Cognitive Function in Former Rugby Union Players Compared with Former Non-Contact-Sport Players and the Impact of Concussion History. *Sports Medicine, 47*(6), 1209-1220.

Ismail, I., Keating, S. E., Baker, M. K., & Johnson, N. A. (2012). A systematic review and meta-analysis of the effect of aerobic vs. resistance exercise training on visceral fat. *Obesity Reviews, 13*(1), 68-91.

Lear, S. A., Humphries, K. H., Kohli, S., Frohlich, J. J., Birmingham, C. L., & Mancini, G. B. (2007). Visceral adipose tissue, a potential risk factor for carotid atherosclerosis: results of the Multicultural Community Health Assessment Trial (M-CHAT). *Stroke*, *38*(9), 2422-2429.

548 Magkos, F., & Yannakoulia, M. (2003). Methodology of dietary assessment in
549 athletes: concepts and pitfalls. *Current Opinion in Clinical Nutrition and Metabolic*
550 *Care*, *6*(5), 539-549.
551
552 Maillard, F. , Pereira, B., & Boisseau, N. (2018). Effect of high-intensity interval
553 training on total, abdominal and visceral fat mass: A meta-analysis. *Sports Medicine,*
554 *48*(2), 269-288.
555
556 Matsuzawa, Y., Shimomura, I., Nakamura, T., Keno, Y., & Tokunaga, K. (1993).
557 Pathophysiology and pathogenesis of visceral fat obesity. *Annals of the New York*
558 *Academy of Sciences, 676,* 270-278.
559
560 Miller, M. A., Croft, L. B., Belanger, A. R., Romero-Corral, A., Somers, V. K.,
561 Roberts, A. J., & Goldman, M. E. (2008). Prevalence of metabolic syndrome in
562 retired National Football League players. *American Journal of Cardiology, 101*(9),
563 1281-1284.
564
565 Murata, H., Oshima, S., Torii, S., Taguchi, M., & Higuchi, M. (2016). Characteristics
566 of body composition and cardiometabolic risk of Japanese male heavyweight Judo
567 athletes. *Journal of Physiological Anthropology*, *35,* 10.
568
569 Nana, A., Slater, G. J., Stewart, A. D., & Burke, L. M. (2015). Methodology review:
570 using dual-energy X-ray absorptiometry (DXA) for the assessment of body
571 composition in athletes and active people. *International Journal of Sports Nutrition*
572 *and Exercise Metabolism, 25*(2), 198-215.

573 Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., …
574 Gakidou, E. (2014). Global, regional, and national prevalence of overweight and
575 obesity in children and adults during 1980-2013: a systematic analysis for the Global
576 Burden of Disease Study 2013. *Lancet*, *384*(9945), 766-781.
577
578 Norton, K., Whittingham, N., Carter, L., Kerr, D., Gore, C., & Marfell-Jones, M.
579 (2006). *Anthropometrica* (K. Norton & T. Olds Eds). Marrickville: Southwood Press.
580
581 Olds, T. (2001). The evolution of physique in male rugby union players in the
582 twentieth century. *Journal of Sports Sciences, 19*(4), 253-262.
583
584 Pickhardt, P. J., Jee, Y., O'Connor, S. D., & del Rio, A. M. (2012). Visceral adiposity
585 and hepatic steatosis at abdominal CT: association with the metabolic syndrome. *AJR:*
586 *American Journal of Roentgenology, 198*(5), 1100-1107.
587
588 Rush, E. C., Freitas, I., & Plank, L. D. (2009). Body size, body composition and fat
589 distribution: comparative analysis of European, Maori, Pacific Island and Asian
590 Indian adults. *British Journal of Nutrition, 102*(4), 632-641.
591
592 Schweitzer, L., Geisler, C., Pourhassan, M., Braun, W., Gluer, C. C., Bosy-Westphal,
593 A., & Muller, M. J. (2015). What is the best reference site for a single MRI slice to
594 assess whole-body skeletal muscle and adipose tissue volumes in healthy adults?
595 *American Journal of Clinical Nutrition, 102*(1), 58-65.
596
597

598 Sedeaud, A., Marc, A., Schipman, J., Tafflet, M., Hager, J. P., & Toussaint, J. F.
599 (2012). How they won Rugby World Cup through height, mass and collective
600 experience. *British Journal of Sports Medicine, 46*(8), 580-584.
601
602 Shen, W., Wang, Z., Punyanita, M., Lei, J., Sinav, A., Kral, J. G., … Heymsfield, S.
603 B. (2003). Adipose tissue quantification by imaging methods: a proposed
604 classification. *Obesity Research, 11*(1), 5-16.
605
606 Swainson, M. G., Batterham, A. M., Tsakirides, C., Rutherford, Z. H., & Hind, K.
607 (2017). Prediction of whole-body fat percentage and visceral adipose tissue mass from
608 five anthropometric variables. *PLoS One, 12*, e0177175.
609
610 Swinburn, B. A., Ley, S. J., Carmichael, H. E., & Plank, L. D. (1999). Body size and
611 composition in Polynesians. *International Journal of Obesity and Related Metabolic*
612 *Disorders, 20*(10), 1178-1183.
613
614 Tchernof, A., & Despres, J. P. (2013). Pathophysiology of human visceral obesity: an
615 update. *Physiological Reviews, 93*(1), 359-404.
616
617 Tonkin, A., Barter, P., Best, J., Boyden, A., Furler, J., Hossack, K. … Clune, E.
618 (2005). National Heart Foundation of Australia and the Cardiac Society of Australia
619 and New Zealand: position statement on lipid management 2005. *Heart, Lung &*
620 *Circulation, 14*, 275-291.
621
622

623 Trexler, E. T., Smith-Ryan, A. E., Defreese, J. D., Marshall, S. W., Guskiewicz, K.
624 M., & Kerr, Z. Y. (2018). Associations between BMI Change and Cardiometabolic
625 Risk in Retired Football Players. *Medicine and Science in Sports and Exercise, 50*(4),
626 684-690.
627
628 Tucker, A. M., Vogel, R. A., Lincoln, A. E., Dunn, R. E., Ahrensfield, D. C., Allen,
629 T. W., … Yates, A. P. (2009). Prevalence of cardiovascular disease risk factors
630 among National Football League players. *JAMA, 301*(20), 2111-2119.
631
632 Verheggen, R. J., Maessen, M. F., Green, D. J., Hermus, A. R., Hopman, M. T., &
633 Thijssen, D. H. (2016). A systematic review and meta-analysis on the effects of
634 exercise training versus hypocaloric diet: distinct effects on body weight and visceral
635 adipose tissue. *Obesity Reviews, 17*(8), 664-690.
636
637 Zemski, A. J., Hind, K., Keating, S. E., Broad, E. M., Marsh, D. J., & Slater, G. J.
638 (2019). Same-day versus consecutive-day precision error of dual-energy X-ray
639 absorptiometry for interpreting body composition change in resistance trained
640 athletes. *Jounral of Clinical Densitometry, 22*(1), 104-114.
641
642 Zemski, A. J., Keating, S. E., Broad, E. M., Marsh, D. J., & Slater, G. J. (2018).
643 Abdominal adiposity distribution in elite rugby union athletes using magnetic
644 resonance imaging. *Sport Sciences for Health,* (in press).
645
646
647

Appendicies

Appendix 1: Three-dimensional images of subcutaneous adipose tissue (SAT; green) and visceral adipose tissue (VAT; red). a) Low VAT (left) vs high VAT (right). b) Caucasian (left; ~21% body fat, ~4700 cm^3 SAT, ~ 600 cm^3 VAT) vs Polynesian (right; 19% body fat, 5900 cm^3 SAT, 800 cm^3 VAT). c) Changes in SAT and VAT in the same athlete over the pre-season.

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Table 1: Descriptive statistics of athletes grouped according to ethnicity.

	Caucasians (n=11)		Polynesians (n=11)			
	Start Pre-Season	**End Pre-Season**	**Start Pre-Season**	**End Pre-Season**	**T-Test *p*-value**	**ANCOVA *p*-value**
Age (years)	22.1 ± 2.4	-	23.5 ± 3.8	-	0.322	-
Surface Anthropometry						
Stature (cm)	189.4 ± 8.7	-	184.1 ± 7.6	-	0.140	-
Body Mass (kg)	101.2 ± 14.2	101.7 ± 14.0	101.8 ± 13.9	100.8 ± 13.6	0.926	0.053
Sum of 7 SF (mm)[#]	66.8 (56.4 to 90.2)	54.7 (47.4 to 71.8)	78.6 (67.1 to 103.7)	65.6 (55.5 to 86.7)	0.150	0.708
WC (cm)	88.7 ± 5.0	87.9 ± 4.5	91.3 ± 6.2	88.5 ± 5.9	0.292	0.007^
BMI (kg/m^2)	28.1 ± 2.1	28.2 ± 2.0	29.9 ± 2.6	29.7 ± 2.7	0.081	0.098
WHt (cm/m^2)	0.47 ± 0.02	0.46 ± 0.02	0.50 ± 0.03	0.48 ± 0.03	0.019*	0.015^
DXA						
Whole body LM (g)	84005 ± 10306	86430 ± 10447	82680 ± 10173	83795 ± 10431	0.767	0.658
Whole body FM (g)	15495 ± 4839	13338 ± 4353	17572 ± 4214	15278 ± 3897	0.296	0.809
Whole body FM (%)	14.7 ± 3.0	12.7 ± 2.7	16.7 ± 2.3	14.7 ± 2.4	0.099	0.515
Android FM (g)	1005 ± 416	818 ± 375	1396 ± 570	1083 ± 462	0.078	0.304
Android FM (%)	14.5 ± 3.8	12.0 ± 3.7	19.4 ± 5.0	15.5 ± 4.5	0.020*	0.351

Gynoid FM (g)	3249 ± 1260	2788 ± 1167	3615 ± 779	3145 ± 786	0.423	0.849
Gynoid FM (%)	17.6 ± 4.2	15.2 ± 4.0	20.2 ± 3.0	17.9 ± 3.2	0.116	0.694
FMI (kg/m^2)	4.3 ± 1.2	3.7 ± 1.1	5.1 ± 1.0	4.5 ± 1.0	0.077	0.915
MRI						
SAT Volume (cm^3)	2279 ± 1014	1888 ± 979	3424 ± 1529	2886 ± 1370	0.068	0.786
VAT Volume (cm^3)#	373 (239 to 649)	206 (181 to 420)	662 (434 to 799)	400 (213 to 547)	0.043*	0.534
Blood Biochemistry						
Fasting Insulin (mU/L)	7.6 ± 4.5	11.0 ± 4.8	7.2 ± 5.3	9.4 ± 4.8	0.717	0.457
Fasting Glucose (mmol/L)	4.2 ± 0.3	4.2 ± 0.7	4.1 ± 0.4	4.3 ± 0.2	0.848	0.452
Total Cholesterol (mmol/L)#	4.3 (3.6 to 4.9)	4.2 (2.8 to 4.7)	4.9 (4.3 to 5.5)	5.0 (4.8 to 5.2)	0.057	0.029^
Triglycerides (mmol/L)#	0.6 (0.3 to 0.7)	0.8 (0.6 to 0.9)	0.8 (0.6 to 1.0)	1.0 (0.8 to 1.2)	0.050*	0.151
HDL-C (mmol/L)	1.4 ± 0.2	1.4 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	0.096	0.936
LDL-C (mmol/L)#	2.2 (1.8 to 2.4)	2.2 (1.8 to 2.5)	2.9 (2.5 to 3.7)	3.1 (2.9 to 3.4)	0.019*	0.122

DXA = dual-energy X-ray absorptiometry; MRI = magnetic resonance imaging; SF = skinfolds; WC = waist circumference; BMI = body mass index; WHt = waist to height ratio; FM = fat mass; LM = lean mass; FMI = fat mass index; SAT = subcutaneous adipose tissue; VAT = visceral adipose tissue; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol

Data presented as mean ± standard deviation

Log transformed, data presented as median (IQR)

Independent T-test – * Significantly different at baseline ($p \leq 0.05$)

ANCOVA – ^ Significant interaction between ethnicity and change over pre-season ($p \leq 0.05$)

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Table 2: Correlations between adiposity measures and blood biochemical markers of cardiometabolic disease risk with magnetic resonance imaging measured SAT and VAT.

	Baseline		Change Over Pre-Season	
	MRI SAT	**MRI VAT**	**MRI SAT**	**MRI VAT**
	***r*#**	***r*#**	***r***	***r*#**
Mass (kg)	0.752**	0.365	0.484*	0.299
Sum 7 Skinfolds (mm)	0.866**	0.639**	0.689**	0.575**
Waist Circumference (cm)	0.842**	0.494*	0.558**	0.578**
BMI (kg/m^2)	0.820**	0.427*	0.496*	0.329
WHt (cm/m^2)	0.525*	0.427*	0.548**	0.588**
Total Fat Mass (kg)	0.953**	0.709**	0.557**	0.496*
Total Fat Mass (%)	0.901**	0.723**	0.473*	0.453*
Android Fat Mass (kg)	0.964**	0.785**	0.625**	0.462*
Android Fat Mass (%)	0.897**	0.834**	0.587**	0.377
Gynoid Fat Mass (kg)	0.881**	0.581**	0.488*	0.491*
Gynoid Fat Mass (%)	0.737**	0.540**	0.420	0.506*
FMI (Fat Mass / Ht (m)^2)	0.939**	0.680**	0.545**	0.500*

Fasting Insulin (mU/L)	0.386	0.263	0.309	0.070
Fasting Glucose (mmol/L)	0.104	0.372	0.272	0.096
Total Cholesterol (mmol/L)	0.435*	0.564**	-0.010	0.302
Triglycerides (mmol/L)	0.705**	0.709**	0.087	0.107
HDL-C (mmol/L)	0.098	-0.044	0.148	-0.067
LDL-C (mmol/L)	0.225	0.411	-0.104	0.078

BMI = body mass index; WHt = waist to height ratio; FMI = fat mass index; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; MRI = magnetic resonance imaging; SAT = subcutaneous adipose tissue; VAT = visceral adipose tissue;

** $p \leq 0.01$, * $p \leq 0.05$

r = Pearson's correlation coefficient; $r^{\#}$ = Spearman's correlation coefficient

$r < 0.1$; small, $0.1 \leq r < 0.3$; moderate, $0.3 \leq r < 0.5$; large, $0.5 \leq r < 0.7$; very large, $0.7 \leq r < 0.9$; almost perfect, $0.9 \leq r < 1.0$; and perfect, $r = 1.0$.

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Table 3: Individual athletes who made meaningful reductions in whole body and regional body composition (> LSC 95%CI – technical error and biological variation) during the pre-season.

		All (n=22)	Ethnicity (n=22)	
			Caucasians (n=11)	Polynesians (n=11)
DXA	Total Fat Mass (g)	17 (77%)	9 (82%)	8 (73%)
	Total Fat Mass (%)	17 (77%)	9 (82%)	8 (73%)
	Android Fat Mass (g)	20 (91%)	10 (91%)	10 (91%)
	Android Fat Mass (%)	19 (86%)	9 (82%)	10 (91%)
	Gynoid Fat Mass (g)	15 (68%)	7 (64%)	8 (73%)
	Gynoid Fat Mass (%)	5 (23%)	2 (18%)	3 (27%)
Surface Anthropometry	Sum of 7 Skinfolds (mm)[a]	21 (95%)	11 (100%)	10 (91%)
	Waist Circumference (cm)[b]	15 (68%)	5 (45%)	10 (91%)
MRI	SAT Volume (cm^3)	19 (86%)	10 (91%)	9 (82%)
	VAT Volume (cm^3)[c]	21 (95%)	11 (100%)	10 (91%)

Data shown are – number of athletes (% of athletes)

DXA = dual-energy X-ray absorptiometry; MRI = magnetic resonance imaging

[a] 1 athlete increased sum of 7 skinfolds (1 Polynesian)

[b] 3 athletes increased waist circumference (2 Caucasian, 1 Polynesian)

[c] 1 athlete increased VAT (1 Polynesian)

682 Figure 1: Pre-season changes in abdominal visceral (VAT) and subcutaneous (SAT)
683 adiposity. Open circles represent Caucasians, closed circles represent Polynesians. Circles
684 joined by lines represent each individual athlete's SAT and VAT values at the start and end
685 of pre-season.