Clinical Study

Metabolic Profiling of Patients Undergoing Elective Aortic Aneurysm Repair Surgery: Correlation with Anaerobic Threshold

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The anaerobic threshold (AT) measured by the cardiopulmonary exercise (CPX) test is becoming an established means of identifying patients at high risk of developing cardiac complications perioperatively. The aim of the present study was to investigate the relationship between AT and the plasma metabolic profile of patients undergoing aortic aneurysm repair surgery to see if an alternative or adjunct to the CPX test could be devised. Plasma was obtained from 15 male patients classified (through preoperative CPX tests) as having high ($\geq 11.0\,\mathrm{mL\,kg^{-1}\,min^{-1}}$) or low ($< 11.0\,\mathrm{mL\,kg^{-1}\,min^{-1}}$) AT before and 1, 2, 24, 48, and 72 hours after elective open aortic aneurysm surgery. Samples were analysed using ^{1}H-NMR spectroscopy coupled with multivariate statistical analysis. Principal components analysis (PCA) and partial least squares discriminant analysis (PLS-DA) distinguished between low- and high-AT patients postoperatively, with high AT patients being more tightly clustered. High AT patients had higher plasma lipid and lower 3-hydroxybutyrate and acetoacetate levels than low AT patients post-operatively. Similar differences were identified preoperatively. ^{1}H-NMR metabolic profiling of plasma has identified molecules whose concentration correlates with AT scores. These may prove a useful biomarker in conjunction with AT in predicting response to major surgical procedures.

1. Introduction

Open aortic aneurysm repair is associated with significant morbidity and mortality [1–5]. An international audit of vascular surgery reported a crude mortality for elective aortic repair of 4.2% [6]. Others have reported mortality rates for elective open aortic repair of 6-7% [7, 8]. The DREAM study reported a combined mortality and severe complication rate of 9.8% for elective open aortic repair [9]. Open aortic repair is associated with a significant systemic inflammatory response syndrome (SIRS) [10]. The more marked this response, the greater (as quantified by the SIRS score) the likelihood of postoperative death [11].

Many methods for the preoperative assessment of the risk of surgery focus on cardiac risk. These include the Lee risk index [12, 13], dobutamine stress echocardiography [14], and radionuclide myocardial perfusion imaging methods [1]. An alternative method of preoperative assessment is cardiopulmonary exercise testing (CPX)[10]. A CPX test is an exercise test in which an individual's functional capacity is quantified in terms of their oxygen uptake in the face of an increasing workload. It offers an integrated assessment of cardiac and respiratory reserve and tissue oxygen utilisation. The anaerobic threshold (AT) is the exercise intensity at which the blood lactate concentration starts to increase during incremental exercise and is quantified in terms of oxygen uptake expressed in $\mathrm{mL\,kg^{-1}\,min^{-1}}$. It has been shown that patients with an AT of less than $11\,\mathrm{mL\,min^{-1}\,kg^{-1}}$ are at increased risk of death following surgery [15–18]. The CPX test may have predictive value because it mimics the postoperative situation: that is, in order to satisfy an increased oxygen demand, an increased respiratory oxygen uptake and cardiac output are required, and thus patients who have a poor oxygen delivery on the ergometer would be expected to have a poor ability to increase cardiac output after surgery [16]. This mechanistic explanation is appealing

but does not take into account the fact that peak oxygen consumption after surgery rarely exceeds VO2 peak even in severely limited patients. An alternative explanation is that a limited functional reserve on CPX testing identifies patients who have deficits that result in a reduced capacity to withstand surgery and a greater likelihood of developing a SIRS response after surgery. This is consistent with the concept of frailty as representing reduced physiological reserve across a number of organ systems [19].

Metabolomics is the technology concerned with the non-targeted identification of all the metabolites in the metabolome [20] and is emerging as a very useful tool for disease diagnosis and biomarker identification [21–24]. By measuring the levels of metabolites, the low-molecular-weight products, and reactants of essential cellular processes, in biofluids like blood plasma, it may be possible to identify metabolic differences, and hence biomarkers from which two or more classes of individuals can be distinguished. Typically, metabolomics studies employ mass spectrometry (MS) or nuclear magnetic resonance (NMR) spectroscopy to obtain metabolic profiles of biofluid samples. NMR spectroscopy has advantages over MS in that it requires minimal sample preparation, is nondestructive to the sample, and produces data with a greater reproducibility. NMR-based metabolomics has been used for biomarker identification in a wide range of fields; recent examples include diagnosis of early-stage epithelial ovarian cancer [25], diagnosis of cerebral infarction [26], identifying severity of chronic liver failure [27], and diagnosis of children with asthma [28]. Of particular significance to the current study is the recent investigation of metabolic changes in traumatic critically ill patients [29]. Some carbohydrate and amino acid levels were found to change in the early part of critical illness, and these observations were in agreement with clinical observations.

The aim of this study was to establish that ^{1}H-NMR-based metabolomics has the potential to identify patients at risk of preoperative deterioration following elective major vascular surgery and to establish a correlation between plasma composition and cardiopulmonary reserve. If such differences are identified, this would support the hypothesis that CPX testing identifies a group of patients who have a metabolic makeup that makes them less able to cope with the stress of surgery. In addition, for patients unable to perform the CPX test such as those whose exercise tolerance is limited by claudication, a new biofluid-accessible biomarker could have real clinical use as a screening test for patients at increased risk of complications.

2. Materials and Methods

2.1. Subjects. This study was approved by the Bradford Research Ethics Committee (REC: 06/Q1201/41). With written informed consent, 15 male patients (mean age 77 (range: 87–68 years)) undergoing elective open aortic aneurysm repair surgery at Leeds General Infirmary, Leeds, UK were recruited for this study. Patients were excluded from the study if they had any of the following: contraindications to CPX testing as listed in the American Thoracic Society

Guidelines [30], an inflammatory aneurysm, or a recent pyrexial illness. The patient demographics are shown in Table 1.

All patients fasted from midnight on the night before surgery and then gradually started to take fluids, and subsequently food, over the next several days. Prior to surgery, the patients underwent incremental CPX testing performed using a cycle ergometer (MedGraphics Breeze suite software-I). Patients were exercised to volitional exhaustion. Whilst aneurysmal disease of the aorta and claudication due to vascular occlusive disease can coexist, no patient in this study was noted to have stopped exercising because of claudication pain. All patients were monitored at rest until the respiratory exchange ratio had settled to less than 1.0. After a period of freewheel cycling, the workload was increased on a smooth ramp at a rate such that if the patient achieved predicted peak exercise, the test would last ten minutes. The test was terminated when the patient could not maintain a cadence of at least 45 rpm on the cycle or indicated that they could not continue. The AT, expressed as oxygen uptake in $mL\,kg^{-1}\,min^{-1}$, was determined using the V-slope method of Wasserman and confirmed by the dual criteria of the nadirs in the ventilatory equivalent for oxygen and end-tidal oxygen. All patients received combined general and epidural anaesthesia and open aortic aneurysm repair. Following echocardiography, all patients had normal left ventricular function apart from one case of mild to moderate left ventricular impairment in each AT group (see Table 1 and text later for the grouping of patients according to high or low AT). Approximately half the patients in each AT group were on statins.

2.2. Plasma Samples. Whole blood samples were collected into lithium-heparinised tubes from each patient. Baseline blood samples were taken at approximately 8 am on the day of surgery and then 1, 2, 24, 48, and 72 hours following the surgery. Plasma samples were obtained by centrifugation of whole blood at 3000 rpm for 10 min and discarding the red blood cell precipitate. Plasma samples were stored in Eppendorf tubes at $-80°C$ until NMR analysis.

2.3. ^{1}H-NMR Spectroscopy. Plasma samples were defrosted by warmth of hands or left at room temperature for 5 minutes before centrifugation at 1270 g for 2 minutes, followed by transfer of 300 μL to an Eppendorf tube. To this, 350 μL of a 0.17% (w/v) solution of the sodium salt of 3-(trimethylsilyl)propionic-2,2,3,3-d$_4$ acid (TSP) (Sigma-Aldrich, Poole, UK) in deuterium oxide (D$_2$O) (Fluorochem, Old Glossop, UK) was added. The mixture was stirred, shaken, and transferred to a 5 mm NMR tube (528PP-WILMAD, Sigma-Aldrich, Poole, UK).

^{1}H-NMR spectra were acquired on a Varian Unity Inova 500 spectrometer, at 21°C. The Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence [RD$-90°-(\tau-180°-\tau)n-$acq] was used to obtain spectra with the signals of macromolecules, such as proteins and lipoproteins, filtered out, leaving only the signals of the low-molecular-weight metabolites of interest. A relaxation delay (RD) of 2 seconds was used,

TABLE 1: Patient cohort used in this study and their AT sores.

| ID | Age/years | AT/mL min^{-1} kg^{-1} | Previous history** | | | Cross clamp time/min |
			HTN	IHD	Diabetes	
			Low-AT group ($n = 7$)*			
1	73	6.1	Y	Y	N	120
2	83	9.8	N	N	N	90
3	87	10.1	N	N	N	70
4	76	10.7	Y	N	N	70
5	82	10.3	Y	Y	Y	63
6	80	9.6	Y	N	N	75
7	71	10.2	Y	Y	Y	75
	79 ± 6[†]	**9.5 ± 1.6**[†]	**5/7**	**3/7**	**2/7**	**80 ± 19**[†]
			High-AT group ($n = 8$)*			
8	68	11.0	Y	Y	N	90
9	71	11.5	Y	N	N	83
10	85	11.7	N	N	N	145
11	79	12.2	N	N	N	60
12	68	13.3	N	N	N	45
13	71	13.7	Y	N	Y	75
14	79	14.0	N	Y	N	75
15	82	14.4	Y	N	N	85
	75 ± 7[†]	**12.7 ± 1.3**[†]	**4/8**	**2/8**	**1/8**	**82 ± 29**[†]

*Low AT was classified as < 11 mL min^{-1} kg^{-1} and high AT as >11 mL min^{-1}kg^{-1}.
**HTN: hypertension; IHD: ischaemic heart disease; N: no; Y: yes.
[†]Average value ± standard deviation.

during which the water resonance was selectively saturated. A spin-spin relaxation time ($2n\tau$) of 450 ms was used, where τ was 1.5 ms and n was 150. 256 transients were collected into 16.384 data points with a spectral width of 6499.84 Hz. An exponential line broadening of 1 Hz was applied to the free induction decays (FIDs) before zero filling in to 64 K. The resulting spectra were phased, baseline corrected, and referenced to TSP at 0.00 ppm.

2.4. NMR Spectral Data Reduction. Spectral data are multivariate, and, thus multivariate statistical analysis techniques, such as principal components analysis (PCA) and partial least squares discriminant analysis (PLS-DA), are required to interpret trends from the data. To remove the effects of peak shifting due to variations in pH and ionic strength, the spectral data were reduced prior to PCA and PLS-DA by "binning," a technique whereby an entire spectrum is divided into "n" equally spaced regions (bins) and the signal area measured within each bin [31]. Data reduction was carried out using VnmrJ 1.1D (Varian Inc., Palo Alto, CA, USA). 225 bins were created over the range −1.00–10.00 ppm, giving a bin width of 0.049 ppm. Labels in figures refer to the centre of a bin. The spectral region between 4.70 and 5.08 ppm was set to zero in order to remove the effects of variation in the efficiency of suppression of the water resonance. The regions 3.21–3.96 ppm (and bins 4.66 and 5.22 ppm) where glucose signals arise were also excluded since glucose concentration can fluctuate greatly amongst samples due to variation in diet of the subjects [32].

To account for the differences in dilution of the plasma samples, and thus, to ensure that the spectra were directly comparable, the areas in each bin for a spectrum were normalised to the total area for that spectrum [33]. Further in the analysis, it became apparent that the postoperative samples varied as a function of the preoperative state of the patient. Therefore, the normalised postoperative samples were normalised a second time, this time by subtracting the preoperative bin values from each of the postoperative bin values.

2.5. Multivariate Statistical Analysis. The binned and normalised NMR data were Pareto scaled and mean centred before PCA was carried out using SIMCA-P+ 11 (Umetrics, Umeå, Sweden) [33]. PCA is an unsupervised technique and so a model is built with no class information about the observations, and only the intrinsic clustering within the dataset is revealed. PCA works by reducing the number of variables in multivariate data into a smaller number of orthogonal variables called principal components (PCs) which describe the maximal variation in the data set. The first principal component (PC) describes the biggest source of variation in the data, the second, the second biggest source, and so on. There could be as many PCs as there are bins; however, many of the PCs will display negligible variation and thus only a few PCs need to be considered when interrogating the features of the sample set. Each observation (in this case, patient) is given a coordinate value for each PC called a "score." Thus by plotting the score

values for each patient along two PCs, the intrinsic clustering between samples is revealed. The variables (bins) are also given a set of coordinates called "loadings," and by plotting these along the same two PCs, the regions of the spectrum responsible for the positioning of the observations in the scores plot can be determined. The quality of the model can be assessed based on the goodness of fit (R^2X value, where X refers to the original variable data matrix) and the model's ability to predict the class membership of new samples (Q^2 value).

In this study, PCA was unable to identify a clear separation between postoperative samples from patients with high and low AT. Therefore, PLS-DA was performed on the postoperative samples after they had been normalised to the preoperative data, as previously described. Unlike PCA, PLS-DA is supervised and thus relies on a parameter that separates the samples into classes, in this case low or high AT. The PLS-DA model was validated using the "leave-one-out method"; one patient (their preoperative and all their postoperative samples) was excluded from the dataset, and a PLS-DA model built from the remaining patients' samples. This model was then used to predict the class membership of the excluded patient. This step was repeated until every patient had been excluded and their class membership predicted. PLS-DA model quality can be assessed by R^2X, Q^2, and R^2Y values, where Y represents the classification parameter(s).

2.6. Univariate Statistical Analysis. Comparisons of the mean values of normalised areas from influential regions of the spectrum were performed using t-tests or Mann-Whitney U tests using SPSS 15.0.0 software (SPSS Inc., Chicago, Illinois, USA), following tests for normality. All P values were adjusted for multiple comparisons using false discovery rate in the software R 2.7.0 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results and Discussion

The anaerobic thresholds for the 15 patients in this study are shown in Table 1. Almost half the patients had an AT < $11 \, \text{mL} \, \text{min}^{-1} \, \text{kg}^{-1}$; these were classified as having a low AT (lAT) for the purposes of this study. Patients with AT > $11 \, \text{mL} \, \text{min}^{-1} \, \text{kg}^{-11}$ were classified as having a high AT (hAT) for the purposes of this study. Whilst AT is clearly a continuous variable, Older et al. [15, 16] demonstrated that patients with an AT of less than $11 \, \text{mL} \, \text{min}^{-1} \, \text{kg}^{-11}$ were at high risk of dying following surgery. This observation has subsequently been confirmed by Wilson and colleagues following an investigation involving over 800 patients [17]. Therefore, for this study, $11 \, \text{mL} \, \text{min}^{-1} \, \text{kg}^{-1}$ was considered the "threshold" value for AT and hence the assignments "lAT" and "hAT."

A PCA model was created containing only the preoperative plasma spectral data which indicated that hAT and lAT patients had differences in their metabolic profiles even before surgery (see Supplementary Material available online at doi:10.5402/2012/341763). However, the differences in the levels of the metabolites permitting discrimination between

Table 2: Metabolites causing the separation between lAT and hAT patients in the PLS-DA scores plot.

Bin(s)/ppm	Metabolite [34]	lAT versus hAT	P-value[a]
1.20 and 2.32	3-Hydroxybutyrate	↑	0.038
2.28	Acetoacetate	↑	0.264
0.92 and 1.30	Lipids	↓	0.037
1.95	Acetate	↓	0.067

[a]For metabolites with signals in more than one bin, the average P value is displayed.

lAT and hAT patients were not found to be statistically significant. Nevertheless, there was indication that there was a difference in the metabolic profiles of patients with lAT and those with hAT, and so this was further investigated. PCA scores plots were created for every postoperative time point versus the preoperative time point, and in each case, a separation between pre- and postoperative samples was shown, irrespective of AT (Figure 1 and see Supplementary Data for metabolites). For all of the PCA models, the third PC revealed a separation between the patients with hAT from those with lAT. The "degree" of separation decreased with time following surgery (Figure 1). Furthermore, the hAT patients were generally more tightly clustered than the lAT patients, suggesting greater metabolic disorder in the lAT patients. The lAT patients appeared to be split into two groups, consisting of patients 1; 6; 5 and 3; 4; 7; 2, respectively. In contrast, the hAT patients were clustered together in one group.

PCA models were created from all patients' plasma samples at each individual postoperative time point to see if a separation between lAT and hAT patients could be achieved. However, these models did not identify a separation between high- and low-AT patients (data not shown), and it was clear that there was inter-sample variation depending on the preoperative state. Therefore, the NMR data for each patient at each time point was normalised to their preoperative sample, and a PLS-DA model ($R^2X = 0.62$, $R^2Y = 0.74$, and $Q^2 = 0.57$) was built from all of the normalised postoperative data. A separation was identified (Figure 2(a)), and the loadings plot (Figure 2(b)) revealed several chemical shift regions that were responsible for this separation (Table 2). hAT patients had significantly higher plasma levels of lipids and lower levels of 3-hydroxybutyrate than lAT patients. hAT patients also had higher acetate and lower acetoacetate than lAT patients, but these were not statistically significant differences.

Since it is a supervised method, PLS-DA requires validation, usually by holding back 33.3% of the data and predicting the class membership of these samples using a PLS-DA model built from the remaining 66.7%. Since the number of patients in this cohort was quite small a "leave-one-out" validation was used instead, revealing a predictability of 60%. To further validate the PLS-DA model, the class of the samples was randomly permutated for 20 times. Figure 3 shows the resultant R^2Y (cum) and Q^2 (cum) values for the models resulting from the permutated data (Figure 3 left) and the original PLS-DA model (Figure 3 right). Both Q^2 (cum) and R^2Y (cum) for the original model were far greater
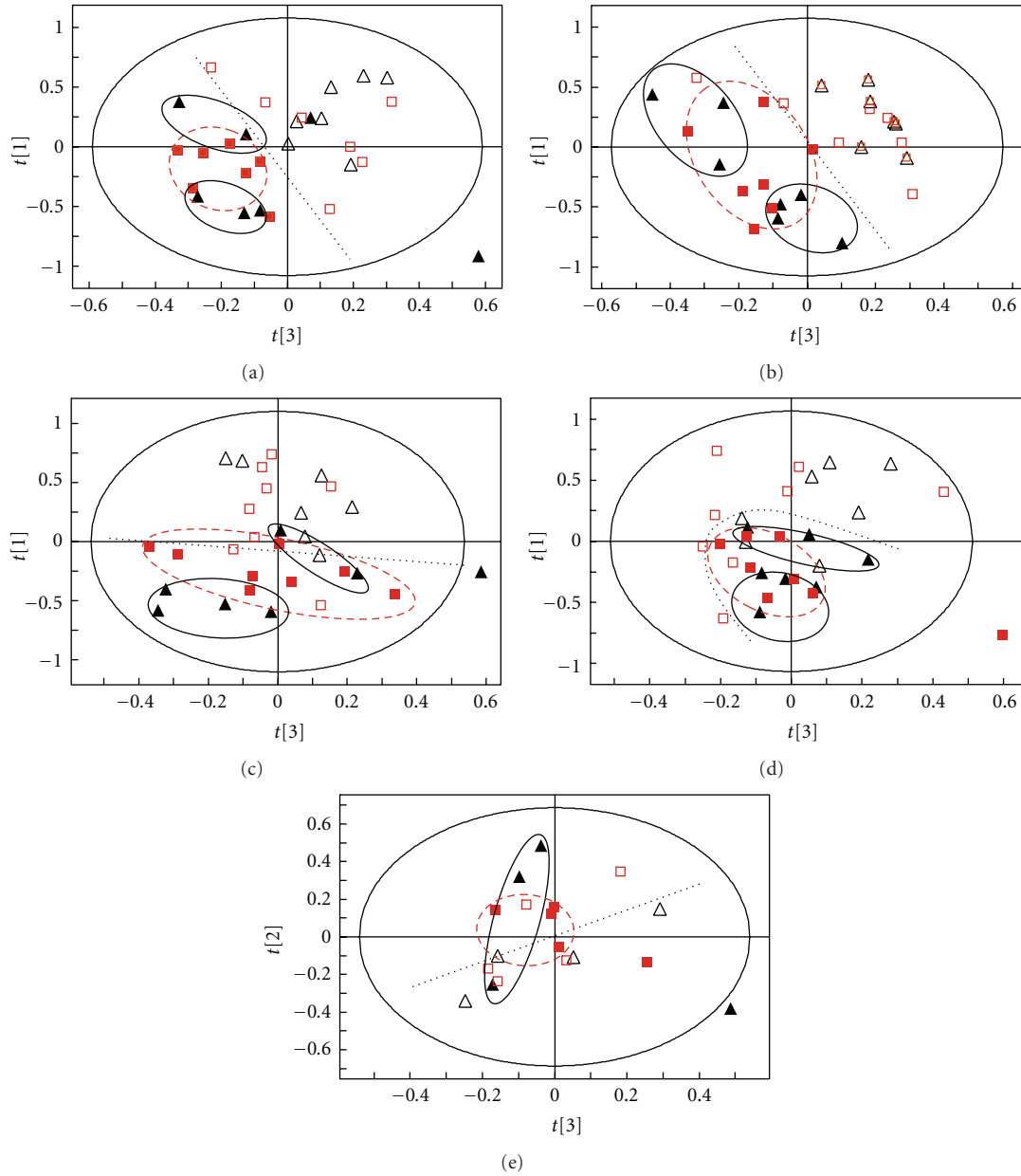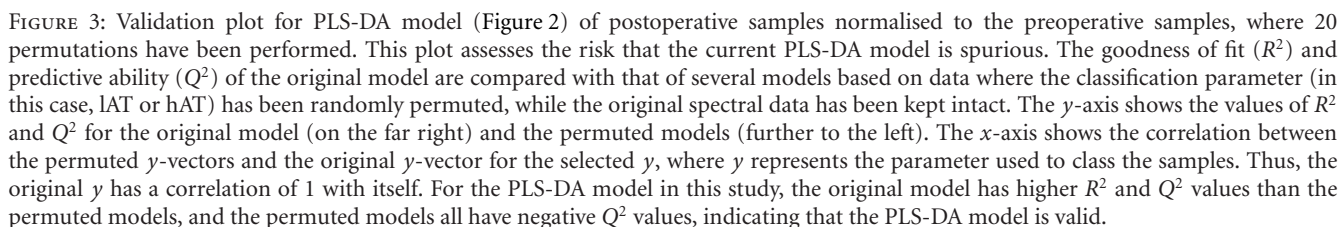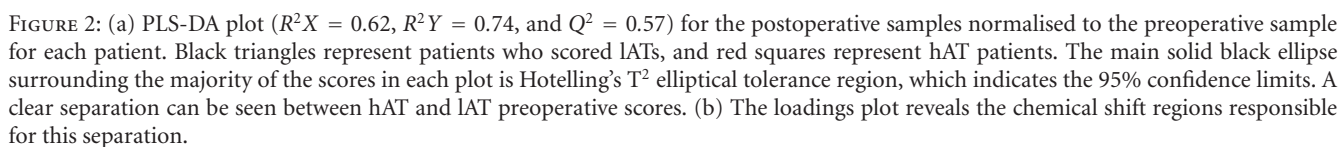
(a)

(b)

(c)

(d)

(e)

FIGURE 1: PCA scores plots from models created using spectral data for some postoperative time point versus the preoperative spectra. Each model had 3 PCs; (a) 1 hour after operation ($R^2X = 0.64$ and $Q^2 = 0.34$); (b) 2 hours after operation ($R^2X = 0.67$ and $Q^2 = 0.46$); (c) 24 hours after operation ($R^2X = 0.65$ and $Q^2 = 0.39$); (d) 48 hours after operation ($R^2X = 0.63$ and $Q^2 = 0.37$); and (e) 72 hours after operation ($R^2X = 0.65$ and $Q^2 = 0.28$). Open shapes are preoperative samples, and filled shapes are postoperative samples. Black triangles represent patients who scored lATs, and red squares represent hAT patients. The dashed line represents the visual divide between pre- and postoperative samples, and the solid line and dashed ovals are for ease of visualisation of grouping of lAT and hAT patients, respectively, and are not statistically relevant. The main solid black ellipse surrounding the majority of the scores in each plot is Hotelling's T^2 elliptical tolerance region, which indicates the 95% confidence limits. These plots show a clear separation between pre- and postoperative samples which diminishes with time. Furthermore, hAT patients are more tightly clustered than the lAT patients, who appear to split into two groups.

than for any of the permutated models, suggesting that the original model was unlikely to be spurious. Furthermore, the Q^2 (cum) intercepted the y-axis below -0.5 indicating that the original PLS-DA model was not overfit and that the permutated data only produced models with very poor predictability.

As mentioned previously, AT is a continuous variable and so separating the patients into groups of high and low AT may be crude. Therefore, an OPLS (orthogonal projections to latent structures) model was built for postoperative samples normalised to the preoperative samples but using the AT values as a continuous vector to regress the plasma

(a)

(b)

FIGURE 2: (a) PLS-DA plot ($R^2X = 0.62$, $R^2Y = 0.74$, and $Q^2 = 0.57$) for the postoperative samples normalised to the preoperative sample for each patient. Black triangles represent patients who scored lATs, and red squares represent hAT patients. The main solid black ellipse surrounding the majority of the scores in each plot is Hotelling's T^2 elliptical tolerance region, which indicates the 95% confidence limits. A clear separation can be seen between hAT and lAT preoperative scores. (b) The loadings plot reveals the chemical shift regions responsible for this separation.

▲ R^2
■ Q^2

FIGURE 3: Validation plot for PLS-DA model (Figure 2) of postoperative samples normalised to the preoperative samples, where 20 permutations have been performed. This plot assesses the risk that the current PLS-DA model is spurious. The goodness of fit (R^2) and predictive ability (Q^2) of the original model are compared with that of several models based on data where the classification parameter (in this case, lAT or hAT) has been randomly permuted, while the original spectral data has been kept intact. The y-axis shows the values of R^2 and Q^2 for the original model (on the far right) and the permuted models (further to the left). The x-axis shows the correlation between the permuted y-vectors and the original y-vector for the selected y, where y represents the parameter used to class the samples. Thus, the original y has a correlation of 1 with itself. For the PLS-DA model in this study, the original model has higher R^2 and Q^2 values than the permuted models, and the permuted models all have negative Q^2 values, indicating that the PLS-DA model is valid.

data against, rather than using, a dummy matrix of variables based on low or high AT. One predictive component and three orthogonal components were produced ($R^2X = 0.60$, $R^2Y = 0.77$, and $Q^2 = 0.61$), and the scores and loadings plots for the predictive component (see Supplementary Data) confirmed a continuous trend in the postoperative plasma compositions of increasing lipids, acetate, and decreasing ketones with increasing AT. Furthermore, similar OPLS models were built for samples taken at each postoperative time point individually to investigate temporal

differences in plasma metabolic profile with respect to AT. Valid models could not be produced for samples taken 1, 2, 48, or 72 hours after surgery, possibly due in part to the reduction in sample numbers in the model, but also as a result of a weaker discrimination between samples of different AT at these time points. A valid model was, however, produced for samples taken 24 hours after surgery ($R^2X = 0.73$, $R^2Y = 0.94$, and $Q^2 = 0.46$) with the scores and loadings plots (Figure 4) confirming the previous findings. This indicates that the largest difference in the metabolic
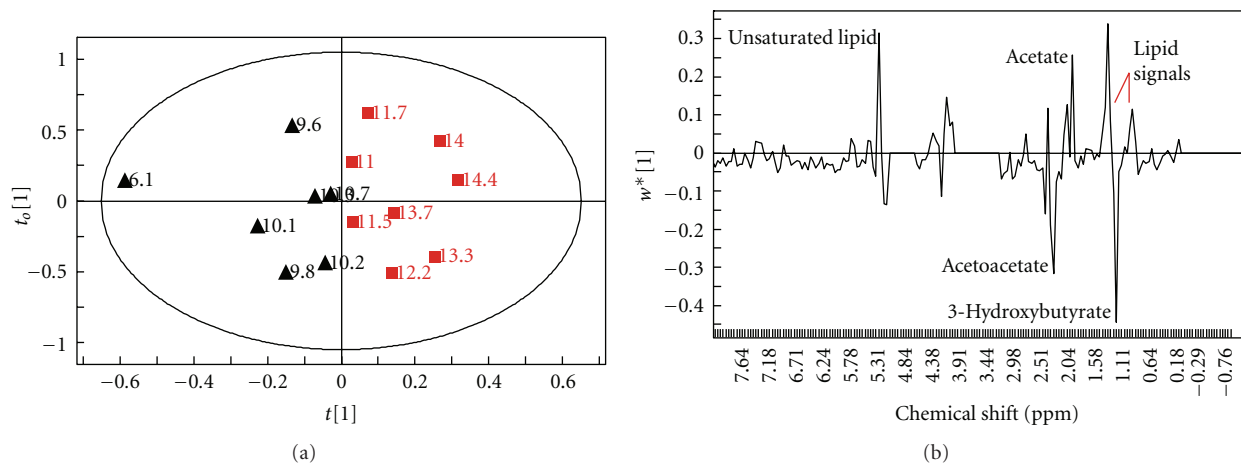
FIGURE 4: (a) Scores plot and (b) loadings line plot for the predictive component ($t[1]$) of the OPLS model created using 24 hours postoperative spectral data normalised to the preoperative data regressed against the AT values of the patients (1 predictive component and 3 orthogonal components, $R^2X = 0.73$, $R^2Y = 0.94$, and $Q^2 = 0.46$). OPLS is an extension of PLS(-DA) where the PLS components are rotated so as to remove any noise in the dataset, producing predictive components, which only account for the variation in the dataset that is related to the response variable. Any variation not related to the response variable is placed into orthogonal components ($t_o[i]$). This makes interpretation of the scores plots easier, since the influence of noise is removed. Black triangles represent patients with AT < 11 mL min^{-1} kg^{-11} (for display only, the model was not built to discriminate according to this cut-off value of AT), and red squares represent patients with AT > 11 mL min^{-1} kg^{-11}. The AT values of the samples are displayed on the plot. The main solid black ellipse surrounding the majority of the scores in each plot is Hotelling's T^2 elliptical tolerance region, which indicates the 95% confidence limits. A clear trend can be seen between the plasma metabolic profile and AT score at 24 hours, with AT values increasing from the left hand side of the plot to the right in a continuous fashion. (b) The loadings plot reveals the chemical shift regions responsible for this trend, and as previously shown, lipids, acetate, and ketones are influential.

profiles of patients with low and high AT occurs 24 hours after surgery, and this difference then diminishes by 48 hours after surgery.

Major surgery is known to cause an increase in reactive oxygen species (ROS) production, and hence the onset of oxidative stress (OS) [15, 35–39]. The occurrence of oxidative stress in aortic aneurysm repair specifically has been recently reviewed [40]. Aivatidi et al.'s findings supported the occurrence of OS during aortic aneurysm repair. It is well established that major surgery is associated with metabolic changes including glycogenolysis, gluconeogenesis, insulin resistance, lipolysis, and the production of ketone bodies [41]. Ketosis is associated with OS. 3-Hydroxybutyrate and its pre-cursor, acetoacetate, have been shown to generate ROS [42–44], and lipids are peroxidised by ROS. These observations are consistent with the lAT patients being more susceptible to oxidative stress during surgery than the hAT patients, since they had higher levels of 3-hydroxybutyrate and acetoacetate, and lower levels of lipids. Developing an understanding of an association between AT and OS could have clinical value, for example, there is evidence in the literature to suggest benefit from antioxidant supplementation such as with vitamin C. Mao et al. [29] used an NMR-based metabolomic approach to compare patients presenting with SIRS, those who had progressed to multiple organ dysfunction syndrome (MODS), and healthy controls. A difference was found between healthy and critically ill patients, though the metabolites causing this were not discussed. When comparing SIRS and MODS patients, the

former had higher levels of sugars and amino acids, whereas the latter had higher levels of lipids, creatinine and lactate [29]. In the present study, plasma lactate, creatinine, sugars, and amino acids were not found to differ between hAT and lAT patients, although, as one would expect, lactate, lipids, and amino acid levels were altered in all patients as a result of the stress response to surgery (see S1) [41]. Plasma lipid levels were influential in the separation of the lAT and hAT patients; hAT patients had higher levels of lipids suggesting a difference in the extent of lipolysis between the two groups. Levels of acetoacetate increase during starvation, and indeed the patients in this study fasted prior to giving the initial plasma samples, and thus, the levels of acetoacetate may reflect the metabolic response preoperative fasting as well as the response to surgery. However, even if levels had risen in both lAT and hAT patients, they were still nevertheless higher in lAT patients compared to hAT patients indicating differences in ketone body production.

PCA models were created for each individual patient to reveal the trajectories of each patient's metabolic profile over time, with "personalized" medicine strategies in mind. An example of a hAT and a lAT patient trajectory is shown in Figure 5. It can be seen that the metabolic profile for the hAT patient 72 hours after surgery is closer to the original composition (time points 72 and 0, resp.) (Figure 5(a)) than is the profile for the lAT patient (Figure 5(b)), suggesting a faster resolution of the metabolic changes induced by the surgical stress response in the hAT patient. This observation, along with the previous finding that lAT patients had more diverse
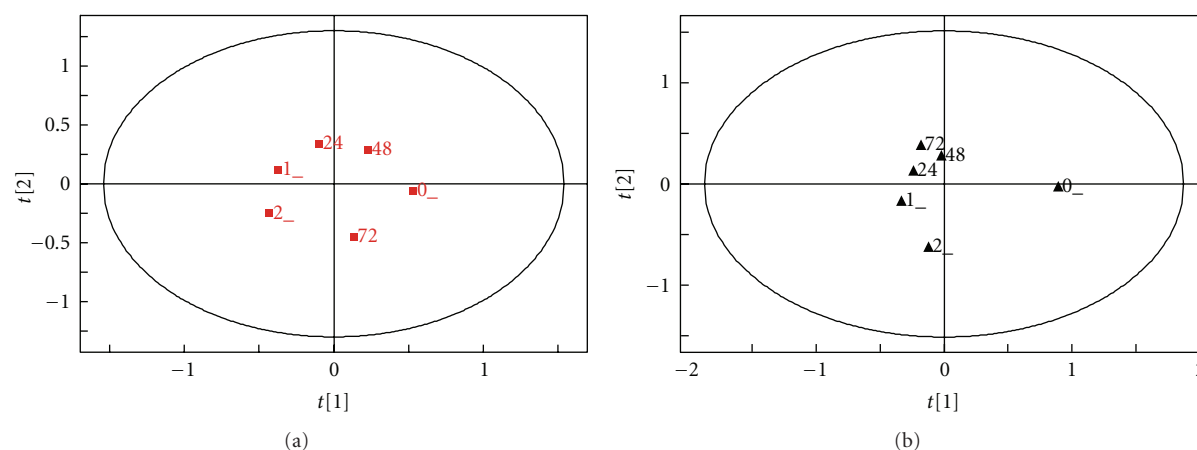
(a)

(b)

FIGURE 5: PCA scores plots of (a) patient 14 (who scored a hAT) (2 PCs, $R^2X = 0.72$ and $Q^2 = 0.17$) and (b) patient 7 (who scored a lAT) (2 PCs, $R^2X = 0.84$ and $Q^2 = 0.19$) to show the trajectory of their individual metabolic profiles over time. The hAT patient appears to have recovered faster than the lAT patient.

metabolic profiles postoperatively than the hAT patients (Figure 1), is consistent with the hypothesis that patients who have a limited cardiopulmonary reserve are less able to meet the metabolic demands of surgery. This is also congruent with the fact that these patients tend to have lower mixed venous oxygen saturation in the postoperative period. Two groups of lAT patients were evident postoperatively, suggesting differing, though not obvious, reasons for their deviation from the hAT postoperative group. The two groups consisted of patients 1; 6; 5 and 3; 4; 7; 2, respectively (Table 1). This initially looks like differentiation within the lAT group simply on the basis of AT. However, patient 168 M had the highest AT of the lAT group, so this division is not so easily explained. Whilst no rationale for this observation can currently be given, it is interesting to note that 18 M, who showed deviation from the other lAT samples between 1 and 24 hours after surgery, had the lowest AT of all the patients. Future studies, with increased sample numbers, may reveal whether this dichotomization is a feature of the oxidative stress status of the patient or some other underlying condition.

As illustrated above, using NMR-based metabolomics in the pursuit of biomarkers, a myriad of metabolites, from different biochemical pathways, can be identified simultaneously, offering a meticulous search. Furthermore, correlations between metabolites can be identified, and it may be the case that discriminations can be made based on combinations of metabolites, rather than single biomarkers. NMR spectroscopy is not as sensitive as other techniques, such as MS, but it has a much greater reproducibility and a higher through-put. Furthermore, most plasma metabolite NMR signals can be assigned, whereas there are still many unknowns in MS-based metabolomics. In the present study, several biomarkers have been identified whose levels vary with AT. Therefore, with further investigation, these potentially could have clinical use in the generation of a "scoring" of risk of developing cardiac complications following major vascular surgery. Ultimately plasma analysis in this way may replace the CPX test, but clearly this is some way off. As

shown in the Supplementary Material, samples from patients with AT at the extremes were clearly distinguished, whereas those with values between 9 and $13 \, \text{mL} \, \text{min}^{-1} \, \text{kg}^{-1}$ were overlapped. However, in situations where a patient is too ill or frail to perform a CPX test, a less invasive blood test could be performed to predict a patient's risk instead. NMR has been used here to identify critical chemical markers which correlate with CPX score (which in turn correlates with risk of postoperative problems). However, it may be that some of these chemicals could be detected by assays more routinely accessible in clinical pathology laboratories. This is an area worthy of further investigation.

4. Conclusions

In conclusion, this pilot study has shown that PCA and PLS-DA of blood plasma metabolic profiles through ^{1}H-NMR spectroscopic analysis have the potential to reveal evidence of OS and how long it takes for recovery following major vascular surgery. We have shown that it is possible to distinguish between patients with high and low AT, from CPX testing, where high-risk/high-score patients show a more stable and rapid recovery than low-risk/score patients. Furthermore, differences in the metabolic profiles of high- and low-risk patients exist even before surgery, and so the methods applied here may have potential applications in predicting an individual patient's risk of developing perioperative organ dysfunction. These methods may prove useful as an adjunct, or in some cases an alternative, to the CPX test. This was a pilot study, and so the sample size is quite small. However, even in this small cohort, some interesting trends have been identified. These findings need firming up by performing this analysis on a large sample cohort.

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