

## ORIGINAL ARTICLE

# Effects of dexmedetomidine, propofol, sevoflurane and S-ketamine on the human metabolome

## *A randomised trial using nuclear magnetic resonance spectroscopy*

Aleksi J. Nummela, Lauri T. Laaksonen, Timo T. Laitio, Roosa E. Kallionpää, Jaakko W. Långsjö, Joonas M. Scheinin, Tero J. Vahlberg, Harri T. Koskela, Viljami Aittomäki, Katja J. Valli, Antti Revonsuo, Mikko Niemi, Markus Perola and Harry Scheinin

**BACKGROUND** Pharmacometabolomics uses large-scale data capturing methods to uncover drug-induced shifts in the metabolic profile. The specific effects of anaesthetics on the human metabolome are largely unknown.

**OBJECTIVE** We aimed to discover whether exposure to routinely used anaesthetics have an acute effect on the human metabolic profile.

**DESIGN** Randomised, open-label, controlled, parallel group, phase IV clinical drug trial.

**SETTING** The study was conducted at Turku PET Centre, University of Turku, Finland, 2016 to 2017.

**PARTICIPANTS** One hundred and sixty healthy male volunteers were recruited. The metabolomic data of 159 were evaluable.

**INTERVENTIONS** Volunteers were randomised to receive a 1-h exposure to equipotent doses ( $EC_{50}$  for verbal command) of dexmedetomidine ( $1.5 \text{ ng ml}^{-1}$ ;  $n = 40$ ), propofol ( $1.7 \text{ } \mu\text{g ml}^{-1}$ ;  $n = 40$ ), sevoflurane (0.9% end-tidal;  $n = 39$ ), S-ketamine ( $0.75 \text{ } \mu\text{g ml}^{-1}$ ;  $n = 20$ ) or placebo ( $n = 20$ ).

**MAIN OUTCOME MEASURES** Metabolite subgroups of apolipoproteins and lipoproteins, cholesterol, glycerides and phospholipids, fatty acids, glycolysis, amino acids, ketone bodies, creatinine and albumin and the inflammatory marker GlycA, were analysed with nuclear magnetic

resonance spectroscopy from arterial blood samples collected at baseline, after anaesthetic administration and 70 min post-anaesthesia.

**RESULTS** All metabolite subgroups were affected. Statistically significant changes vs. placebo were observed in 11.0, 41.3, 0.65 and 3.9% of the 155 analytes in the dexmedetomidine, propofol, sevoflurane and S-ketamine groups, respectively. Dexmedetomidine increased glucose, decreased ketone bodies and affected lipoproteins and apolipoproteins. Propofol altered lipoproteins, fatty acids, glycerides and phospholipids and slightly increased inflammatory marker glycoprotein acetylation. Sevoflurane was relatively inert. S-ketamine increased glucose and lactate, whereas branched chain amino acids and tyrosine decreased.

**CONCLUSION** A 1-h exposure to moderate doses of routinely used anaesthetics led to significant and characteristic alterations in the metabolic profile. Dexmedetomidine-induced alterations mirror  $\alpha_2$ -adrenoceptor agonism. Propofol emulsion altered the lipid profile. The inertness of sevoflurane might prove useful in vulnerable patients. S-ketamine induced amino acid alterations might be linked to its suggested antidepressive properties.

**TRIAL REGISTRATION** ClinicalTrials.gov identifier: NCT02624401

Published online 23 August 2021

From the Department of Peri-operative Services, Intensive Care and Pain Medicine, Turku University Hospital (AJN, LTL, TTL, REK, JMS, KJV, HS), Department of Internal Medicine, Turku University Hospital, Turku (AJN), Turku PET Centre, University of Turku and Turku University Hospital (LTL, HS), Department of Psychology and Speech-Language Pathology and Turku Brain and Mind Center, University of Turku, Turku (REK, KJV, AR), Department of Intensive Care, Tampere University Hospital, Tampere (JWL), Department of Clinical Medicine, Biostatistics, University of Turku and Turku University Hospital, Turku (TJV), Nightingale Health Ltd, Helsinki, Finland (HTK, VA), Department of Cognitive Neuroscience and Philosophy, School of Bioscience, University of Skövde, Skövde, Sweden (KJV, AR), Department of Clinical Pharmacology, University of Helsinki and Helsinki University Hospital (MN), Individualized Drug Therapy Research Program, Faculty of Medicine, University of Helsinki (MN), Finnish Institute for Health and Welfare (MP), Research Program for Clinical and Molecular Metabolism, Faculty of Medicine, University of Helsinki, Helsinki (MP), and Integrative Physiology and Pharmacology, Institute of Biomedicine, University of Turku, Turku, Finland (HS)

Correspondence to Aleksi J. Nummela, Department of Internal Medicine, Turku University Hospital, 20521 Turku, Finland

E-mail: aljunu@utu.fi

0265-0215 Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the European Society of Anaesthesiology and Intensive Care.

DOI:10.1097/EJA.0000000000001591

This is an open access article distributed under the Creative Commons Attribution License 4.0 (CCBY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

## Introduction

Metabolomics has provided promising results in the fields of early diagnosis, biomarker discovery and stratification of disease risk-groups.<sup>1–4</sup> Pharmacometabolomics, a relatively new research direction, uses large scale data-capturing methods to uncover shifts in the metabolic profile induced by pharmacotherapy. Further analysis of these changes in biochemical pathways and identification of baseline predictors of patient response via biomarker acquisition may eventually offer an opportunity to enhance early prediction of treatment outcomes, to reveal novel mechanisms of drug action and to identify metabolic pathways contributing to drug response phenotypes.<sup>2,5–8</sup> Ultimately, pharmacometabolomics might enable the discovery of novel tools for clinical decision-making, the optimisation of personalised medical therapy and precision medicine.

The pharmacometabolomic effects of anaesthetic agents remain insufficiently documented. The effects of dexmedetomidine on insulin secretion, S-ketamine on circulating catecholamines and propofol on triglyceride levels are examples of pharmacological attributes that may have interesting metabolic ramifications.<sup>9–21</sup> In this exploratory study, the aim was to discover whether four routinely used anaesthetics, dexmedetomidine, propofol, sevoflurane and S-ketamine, cause acute alterations in the human metabolic profile in the absence of confounding factors present in clinical anaesthesia and surgery. An

infographic summarising the study design, metabolomic analysis and results is shown in Fig. 1.

## Materials and methods

### Ethics

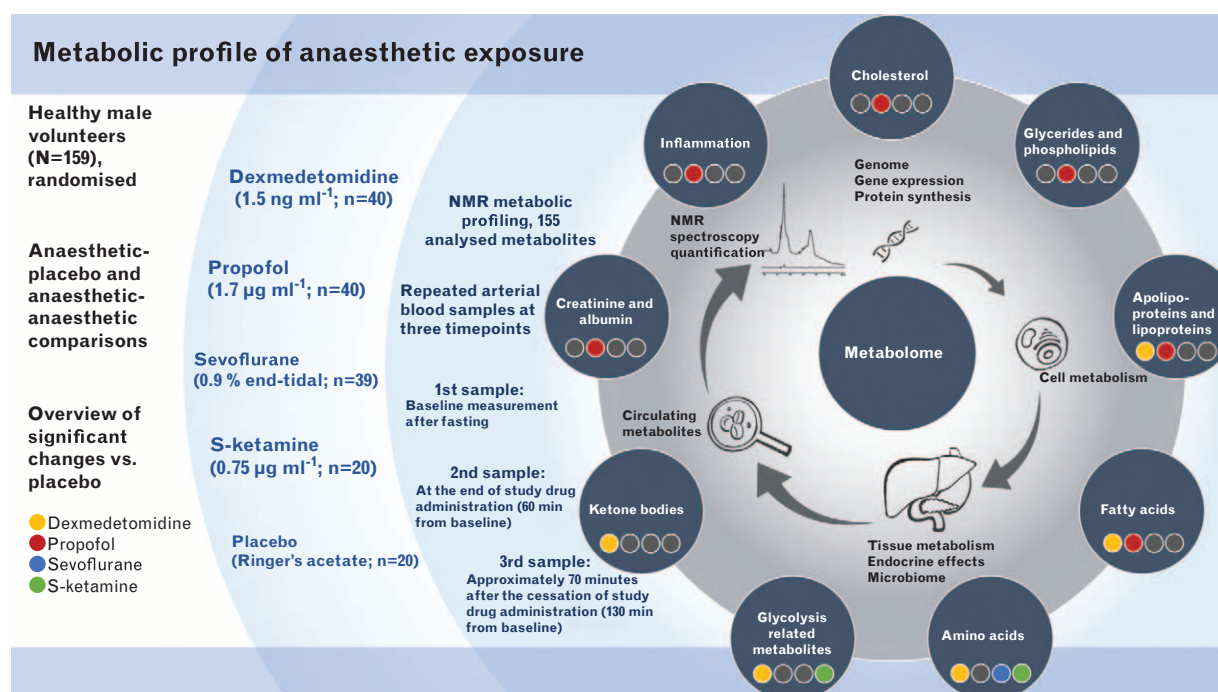
Approval for this study (LOC-2016, EudraCT 2015-004982-10, ClinicalTrials.gov Identifier NCT02624401) was provided by the Ethics Committee of Hospital District of Southwest Finland, Turku, Finland on 15 December 2015.

### Trial design and participants

This randomised, open-label, controlled, parallel group, phase IV clinical drug trial was conducted at Turku PET Centre, University of Turku, Finland, as a part of 'The Neural Mechanisms of Anaesthesia and Human Consciousness' project. A detailed description of the study methods has been published previously in an article comparing the effects of anaesthetic agents on regional cerebral glucose metabolism.<sup>22</sup>

One hundred and sixty healthy, American Society of Anaesthesiologists class I (ASA I) male volunteers were randomised to receive dexmedetomidine (Dexdor 100 µg ml<sup>-1</sup>, Orion Pharma, Espoo, Finland; *n* = 40), propofol (Propolipid 10 mg ml<sup>-1</sup>; Fresenius Kabi, Bad Homburg, Germany; *n* = 40), sevoflurane (Sevoflurane 100%, Abbvie, North Chicago, Illinois, U.S.A; *n* = 40),

Fig. 1 Infographic for the study



The circulating metabolic profile arises from a complex interplay of biochemical pathways reflecting changes on many levels: From gene expression to cell and tissue metabolism.

S-ketamine (Ketanest-S 25 mg ml<sup>-1</sup>, Pfizer, NY, U.S.A;  $n = 20$ ) or placebo (Ringer's acetate,  $n = 20$ ). The inclusion criteria have been described earlier.<sup>22</sup> Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

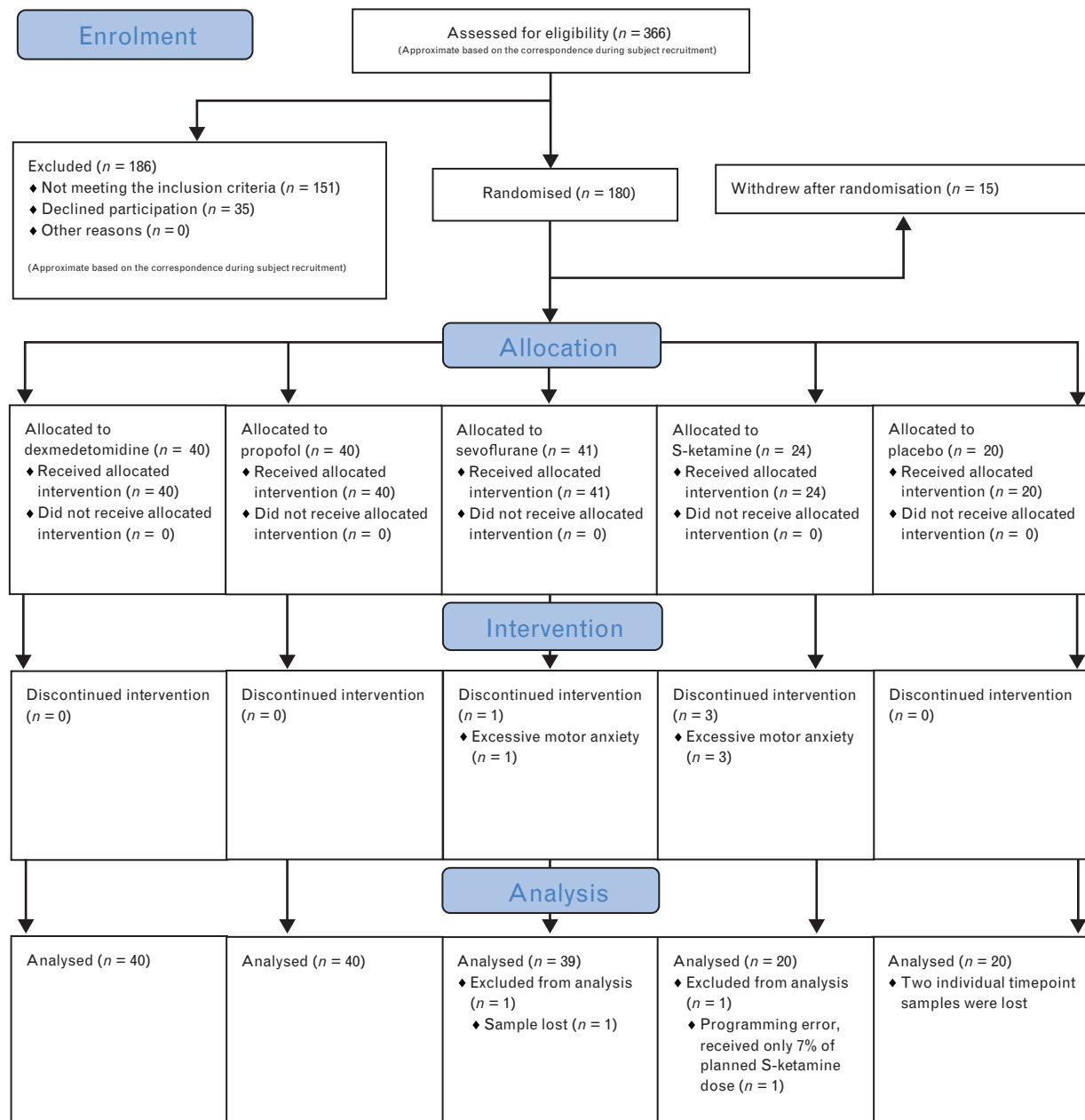
Only male participants were studied because of radiation exposure related to a subsequent PET study. Due to the exploratory nature of the study, a formal power analysis was not considered applicable. Randomisation was performed using balanced permuted block sizes of 16 as

previously described.<sup>22</sup> Fig. 2 shows the Consort Flow diagram for the study participants.

### Anaesthetic protocol and monitoring

All participants fasted from midnight until time of anaesthesia. Intake of alcohol, caffeine and medications were prohibited preceding anaesthesia. The duration of the anaesthetic administration protocol was 60 min; details have been published earlier.<sup>22</sup> Intravenous anaesthetics were administered using target-controlled infusion with a

Fig. 2 Consort flow diagram for participants in the study



CONSORT flow diagram showing each stage of the randomised-controlled-trial, enrolment, allocation, intervention and analysis.

Harvard 22 syringe pump (Harvard Apparatus, South Natick, Massachusetts, USA) and Stanpump software ([www.opentci.org/code/stanpump](http://www.opentci.org/code/stanpump)). Previously reported pharmacokinetic parameters were used (Domino, Talke, March-models).<sup>23–25</sup> Sevoflurane was administered and monitored using a Drager Primus anaesthesia workstation (Dragerwerk AG & Co KGaA, Lübeck, Germany). The details of participant preparation and monitoring have been described earlier.<sup>22</sup>

The target concentrations (EC<sub>50</sub> for verbal command) were based on previous studies: 1.5 ng ml<sup>-1</sup> for dexmedetomidine, 1.7 µg ml<sup>-1</sup> for propofol, 0.75 µg ml<sup>-1</sup> for S-ketamine and end-tidal target of 0.9% for sevoflurane.<sup>26–28</sup> The arterial blood concentrations of the intravenous anaesthetics were measured off-line and the end-tidal concentration of sevoflurane was monitored continuously during anaesthetic administration.

### Blood sampling and metabolomic analysis

Arterial blood samples for metabolomic analysis were collected before anaesthetic administration (timepoint 1), at the end of 60 min of anaesthetic administration (timepoint 2) and 70 min after the cessation of anaesthetic administration (timepoint 3). A 9 ml whole blood EDTA sample was collected at each timepoint. The blood samples were protected from light and placed on ice immediately after sampling. Within 30 min, plasma was separated using cold centrifugation (+4°C) and divided into amber tubes (Matrix 1.0 mL 2D Screw tubes Amper PP; Thermo Scientific, USA). Amber tube samples were immediately frozen at -20°C and the samples were transferred to -70°C within the same day keeping the temperature under -70°C by means of dry ice. Sampling, storage and transfer of the metabolomics samples were conducted according to the specifications of the company responsible for metabolite quantification (Nightingale Health, Helsinki, Finland).

Metabolic biomarkers were quantified from plasma samples using high-throughput nuclear magnetic resonance (NMR) spectroscopy (Nightingale Health Ltd, Helsinki, Finland). The NMR metabolomics platform is based on proton NMR spectroscopy, wherein each molecule with hydrogen atoms gives a characteristic spectral signal. This signal is of a distinctive shape, which permits the identification of molecules, and the signal area is proportional to the concentration of the molecule in the sample. Overlaps of individual molecular signals and quantification of absolute concentrations are handled via proprietary data processing methods. This method provides simultaneous quantification of routine lipids, lipoprotein subclass profiling, fatty acid composition and various low-molecular weight metabolites, including amino acids, ketone bodies and glycolysis-related metabolites in molar concentration units. The metabolic profile obtained can be considered a targeted metabolic profile as specific, predetermined metabolites are quantified. The

company uses proprietary data processing methods but a more detailed description of the current NMR methodology has been reported previously.<sup>4,29</sup>

From each sample, 146 metabolite markers and nine ratios were analysed or calculated. The measured biomarkers included 101 lipoprotein measures, 37 lipid-related markers (including 16 fatty acid, nine cholesterol, nine glycerides and phospholipids and three apolipoprotein measures), concentrations of eight amino acids, three glycolysis-related metabolites, three ketone bodies, creatinine and albumin and an inflammatory marker glycoprotein acetylation. The metabolite markers within each of the aforementioned metabolite subgroups are illustrated in the forest plots in Appendix A, <http://links.lww.com/EJA/A603>.

### In vitro analyses

Because of the lipid emulsion formulation of propofol and due to extensive changes observed in the lipoprotein markers during propofol infusion in vivo, as a quality control measure, in vitro analyses were carried out to ensure that the lipid formulation of propofol had no direct effect on NMR quantification of metabolite markers in vitro at propofol target concentration of 1.7 µg ml<sup>-1</sup>. The same NMR instrumentation as in the main analyses was used (Nightingale Health). In vitro analyses were conducted using pooled human serum samples that had been previously prepared from whole blood samples collected in lithium heparin tubes and stored at -80°C, similar to the main analysis. Propofol was added to pooled samples in incremental concentrations (1.75 to 100 µg ml<sup>-1</sup>). Metabolite concentrations were measured and compared with a blank pooled sample (without added propofol) and with each added propofol concentration. In addition, the mean changes in very low-density lipoprotein particle (VLDL) markers in the propofol group were compared with the VLDL changes in the in vitro experiment to assess the possibility of soybean oil accumulation during continuous infusion. The results of the in vitro analysis are described in detail in Appendix B, <http://links.lww.com/EJA/A604>.

### Statistical analysis

Logarithmic transformation was performed for metabolites with skewness more than 1 (42% of all metabolites). All metabolites were scaled to baseline standard deviation (SD) to enable comparisons across metabolites with different units. The mean group difference in SD change units is referred to as the standard deviation score (SDS) and was chosen instead of the Z-score to allow easy comparison to earlier studies. Zero-values, including values under the detection limit of the NMR spectroscopy method, were omitted from the analysis (4% of all measured values). The statistical analysis of metabolite markers was performed using repeated measures analysis of variance (ANOVA) with each metabolite marker as

outcome and time as a within factor and group as a between factor.<sup>30</sup> Because all metabolites were analysed using separate ANOVA models, there are no assumptions concerning the dependency between metabolites. The mean differences in SD changes (95% CI) between groups for all metabolites were estimated from a repeated measures model using group by time interaction effect. The anaesthetic-placebo and anaesthetic-anaesthetic group differences in SD changes were estimated between timepoints 1 vs. 2 and 1 vs. 3. To account for multiple testing (155 metabolites, 10 pairwise group comparisons and two timepoint comparisons) the observed *P* values were Bonferroni corrected by factor 3100 such that an alpha threshold of 0.05 remained. Data are expressed as SDS (95% CI) between timepoints 1 vs. 2, if not otherwise stated. Statistical analyses were carried out with SAS software (version 9.4; SAS Institute Inc., Cary, North Carolina, USA).

For data visualisation, forest plots and line graphs were created using R (Version 1.1.383, <https://www.R-project.org/>) ggplot2 function (Version 3.2.1, <https://ggplot2.tidyverse.org/>). The infographic was created using Microsoft PowerPoint (version 16.16.18). The cross-correlations of the analytes were calculated based on the current data of three timepoints of 159 participants (excluding two missing timepoint samples in the placebo group, *n* = 475, Appendix C, <http://links.lww.com/EJA/A605>). The colour coding represents Spearman's correlation coefficients.

## Results

The baseline characteristics of the volunteers are summarised in Table 1. The study was completed as planned and no significant changes in the vital signs were observed. Anaesthetic concentrations were stable in all groups. The mean concentrations of propofol and sevoflurane approximated the predicted concentrations, but dexmedetomidine and S-ketamine were approximately 40% higher than targeted (Fig. 3). The metabolomics data of 159 participants were evaluable, as all timepoint samples of one in the sevoflurane group were lost, as were two individual timepoint samples in the placebo group (Fig. 4).

In anaesthetic-placebo comparisons (timepoints 1 vs. 2 and 1 vs. 3), significant changes were observed in all metabolite subgroups (Fig. 2). Of the 155 analysed metabolites there were significant changes vs. placebo in 17 (11.0%), 64 (41.3%), one (0.65%) and six (3.9%) of

the analytes in the dexmedetomidine, propofol, sevoflurane and S-ketamine groups, respectively. The majority of these changes remained significant in one or more anaesthetic-anaesthetic comparisons (Appendix A, <http://links.lww.com/EJA/A603>). Due to the large number of significant changes, up to three metabolites in each anaesthetic group showing the largest statistically significant SDS change (timepoints 1 vs. 2) are shown in detail in Table 2, Fig. 4 and Fig. 5. All analysed biomarker changes in all inter-group comparisons are shown in Appendix A, <http://links.lww.com/EJA/A603>. A heatmap containing cross-correlations of measured metabolites is provided in Appendix C, <http://links.lww.com/EJA/A605>.

In the dexmedetomidine group, strongest observed changes were the elevation of glucose and concomitant decrease in ketone bodies (Table 2). Changes in HDL composition and a decrease in very large HDL concentration of -0.29 SDS (95% CI -0.42 to -0.17), *P* = 0.02 were observed. The anaesthetic-placebo changes in glucose, 3-hydroxybutyrate and very large HDL phospholipid and free cholesterol content were significant in all inter-group comparisons.

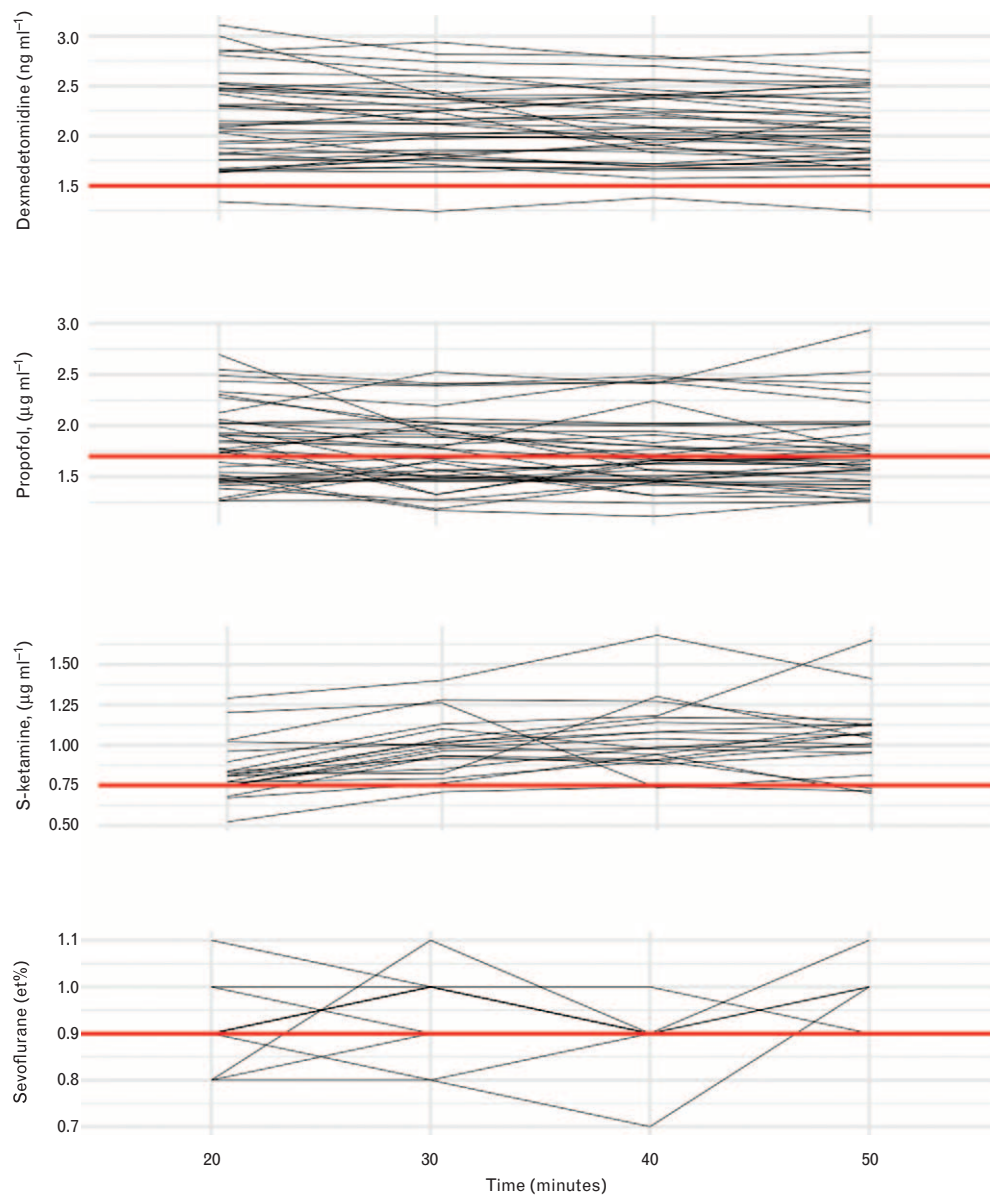
Extensive changes in the lipid profile were observed in the propofol group. Strongest increase was observed in saturated fatty acids to total fatty acids ratio (SFA\_FA), very large VLDL free cholesterol content (XL\_VLDL\_FC) and very large HDL triglyceride content (Table 2). Unsaturated fatty acids decreased by -0.45 SDS (95% CI -0.60 to -0.30), *P* < 0.001 and saturated fatty acids (SFA) increased by 0.54 SDS (95% CI 0.37 to 0.70), *P* < 0.001. Significant changes in lipoprotein concentration and content were observed in a range of lipoprotein particles sizes (Appendix A, <http://links.lww.com/EJA/A603>). Only a modest increase in serum total triglyceride level (Serum\_TG) 0.32 SDS (95% CI 0.21 to 0.42), *P* < 0.001 was observed. The inflammatory marker glycoprotein acetylation (GlycA) increased by 0.37 SDS (95% CI 0.21 to 0.53), *P* = 0.023. Thirty-seven metabolites showed significant changes in all inter-group comparisons in metabolite subgroups of lipoproteins, fatty acids and glycerides and phospholipids (Appendix A, <http://links.lww.com/EJA/A603>).

In the sevoflurane group, only the increase in alanine was significant and was 0.6 SDS (95% CI 0.4 to 0.81), *P* < 0.001.

**Table 1** Baseline characteristics of volunteers

	n	Height (cm)	Weight (kg)	BMI	Age (years)
Dexmedetomidine	40	179.1 ± 6.5	77.3 ± 10.8	24.1 ± 2.9	24.7 [20 to 30]
Propofol	40	180.5 ± 6.0	77.6 ± 11.1	23.8 ± 3.3	23.4 [18 to 28]
Sevoflurane	39	179.7 ± 7.2	79.5 ± 9.7	24.6 ± 2.8	24.4 [19 to 30]
S-ketamine	20	182.7 ± 5.4	79.9 ± 10.6	23.9 ± 3.0	23.4 [20 to 30]
Placebo	20	182.4 ± 8.8	82.9 ± 14.2	24.8 ± 2.6	23.1 [20 to 28]

Data are given as mean ± SD or mean [range].

**Fig. 3** Individual measured concentrations of the four anaesthetics: Dexmedetomidine, propofol, sevoflurane, S-ketamine

The study drugs were administered for 60 minutes, but their concentrations were measured at 20, 30, 40 and 50 minutes. Targeted concentrations are depicted with red lines. Mean measured concentrations have been published earlier.<sup>22</sup>

In the S-ketamine group, strongest changes were observed in glucose, lactate and leucine. Glucose and lactate increased, while leucine decreased (Table 2). Isoleucine decreased by -0.89 SDS (95% CI -1.25 to -0.54),  $P=0.006$ , tyrosine by -0.89 SDS (95% CI -1.27 to -0.52),  $P=0.017$  (timepoints 1 vs. 3). The decrease in leucine was significant in all inter-group comparisons.

#### In vitro analyses

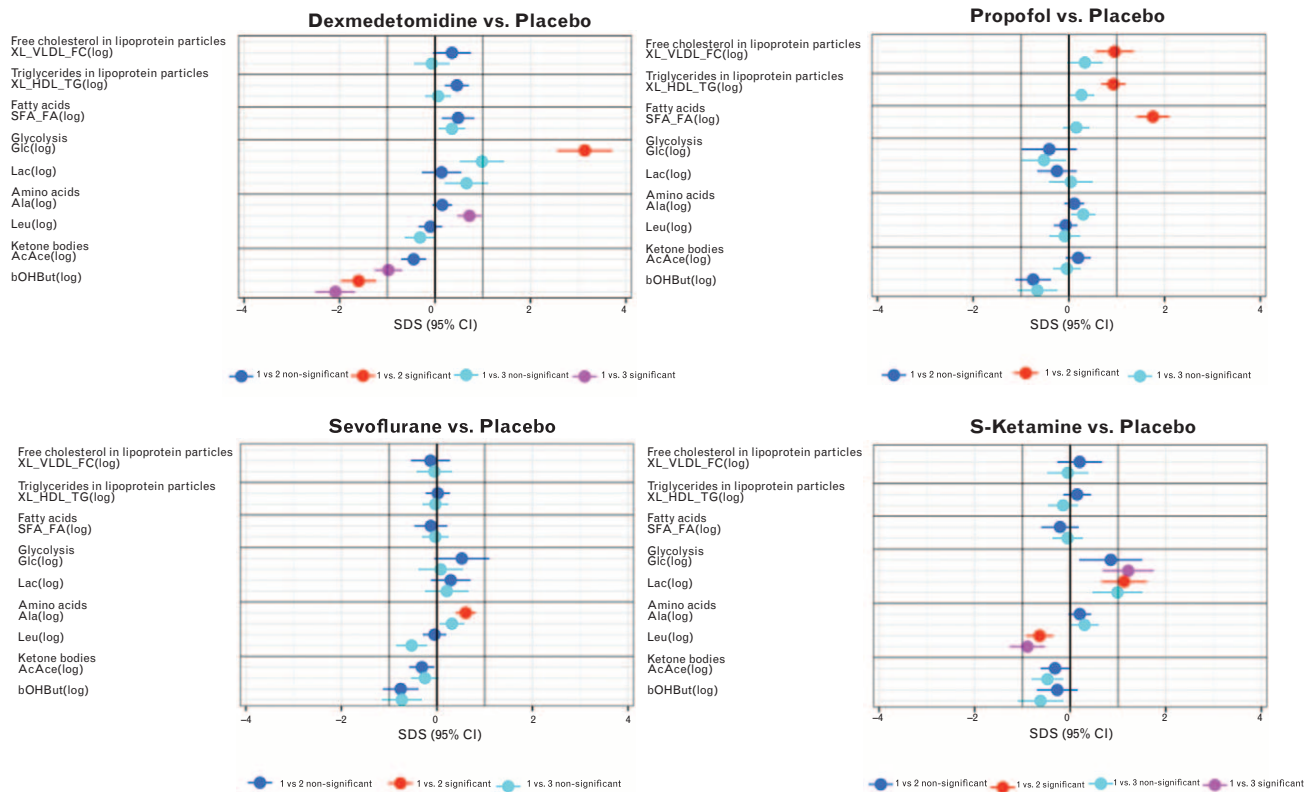
Briefly, the in vitro analysis showed that propofol emulsion, when directly added to baseline samples at concentrations corresponding to the target concentration of  $1.7 \mu\text{g ml}^{-1}$ , caused only minor changes in the

metabolic profile. Interestingly, the in vivo propofol infusion with a target concentration of  $1.7 \mu\text{g ml}^{-1}$  resulted in a change in VLDL markers corresponding to the levels observed at propofol concentration of  $17.5 \mu\text{g ml}^{-1}$  in vitro. The detailed results of in vitro analyses are described in Appendix B, <http://links.lww.com/EJA/A604>.

#### Discussion

In this study, we evaluated the metabolic profiles of anaesthetic exposure to equipotent doses ( $\text{EC}_{50}$  for verbal command) of dexmedetomidine, propofol, sevoflurane and S-ketamine in healthy men using NMR

Fig. 4 Forest plots of nine selected anaesthetic-placebo comparisons



Data are reported as SDS and 95% confidence intervals. The vertical lines depict 0 and  $\pm 1$  SDS thresholds. Positive values (to the right) depict an increase in metabolite concentration compared with placebo, while negative values (to the left) represent a decrease compared with placebo. The colour coding represents the changes during anaesthetic administration (in timepoints 1 vs. 2) and from baseline to 70 min after anaesthetic administration (in timepoints 1 vs. 3). Statistically significant changes are highlighted in red and purple (timepoints 1 vs. 2 and 1 vs. 3, respectively). Logarithmic transformation was carried out for skewed metabolites, these metabolites are marked with (log). Ala, alanine; AcAce, acetoacetate; bOHBut, 3-hydroxybutyrate; Glc, glucose; Lac, lactate; Leu, leucine; SFA\_FA, ratio of saturated fatty acids to total fatty acids; XL\_HDL\_TG, triglycerides in very large HDL particles; XL\_VLDL\_FC, free cholesterol in very large VLDL particles. Forest plots with all metabolites and all inter-group comparisons are shown in Appendix A, <http://links.lww.com/EJA/A603>.

spectroscopy. Interestingly, marked alterations in the metabolic profiles of anaesthetics vs. placebo were revealed, regardless of moderate dosing and a relatively short anaesthetic exposure. Furthermore, the metabolic profiles of four commonly used anaesthetics were quite different.

The changes in the metabolic profile of dexmedetomidine were mainly seen in glucose and ketone bodies. Dexmedetomidine increased glucose concentration 29% from baseline. Changes in HDL composition and a decrease in very large HDL concentration were observed. Increased glucose and concomitantly decreased ketone bodies in fasted individuals reflect dexmedetomidine-induced inhibition of insulin secretion in response to  $\alpha_2$ -adrenoceptor agonism.<sup>15,16</sup> It is possible that the alterations in HDL markers too (Appendix A, <http://links.lww.com/EJA/A603>) are linked to ( $\alpha_2$ -adrenoceptor agonism, as selective alpha adrenoceptor blockade is known to increase HDL levels.<sup>31</sup>

Propofol induced a subtle increase in the inflammatory marker GlycA, the concentration of which was 3.8% higher in the propofol group at timepoint 2 when compared with placebo. GlycA is a complex NMR signal arising from the N-acetyl groups in circulating glycoproteins that are involved in the acute phase response. Chronically elevated GlycA has been associated with a gene network enriched for neutrophil functions, elevated levels of inflammatory cytokines, long-term risk of serious infection and all-cause mortality.<sup>32,33</sup> However, little is known about acute alterations in GlycA.<sup>34</sup> Our interpretation of the results is that even though GlycA elevation seems mechanistically interesting, the magnitude of GlycA increase in response to short-term propofol administration is small and unlikely to have clinical implications.<sup>34</sup>

Propofol induced marked changes in the lipid profile, and surprisingly, only a modest increase in total triglycerides was observed. In the propofol group, serum total triglycerides increased 8.1% (timepoints 1 vs. 2). As a

Table 2 Summary of absolute and SDS changes in selected metabolites

	Timepoint			Timepoint	Difference in SDS			P
	1	2	3		1 vs. 2	1 vs. 3	1 vs. 2	
<b>Deemedetomidine</b>								
Glc (mmol l <sup>-1</sup> )	4.3 ± 0.35	5.56 ± 0.61	4.6 ± 0.38	4.11 ± 0.30	4.08 ± 0.22	3.14 (2.56 to 3.71)	0.98 (0.52 to 1.45)	<0.001
bOHBut (mmol l <sup>-1</sup> )	0.18 ± 0.1	0.13 ± 0.05	0.12 ± 0.05	0.3 ± 0.2	0.32 ± 0.18	-1.60 (-1.97 to -1.23)	-2.09 (-2.5 to -1.67)	<0.001
AcAce (mmol l <sup>-1</sup> )	0.039 ± 0.015	0.04 ± 0.016	0.035 ± 0.016	0.054 ± 0.023	0.062 ± 0.028	-0.45 (-0.71 to -0.19)	-0.98 (-1.27 to -0.69)	<0.001
<b>Propofol</b>								
SFA_FA (%)	35.2 ± 1.75	37.7 ± 1.74	35.4 ± 1.62	35.2 ± 1.1	35.3 ± 0.99	1.76 (1.42 to 2.1)	0.15 (-0.12 to 0.43)	<0.001
XL_VLDL_FC (mmol l <sup>-1</sup> )	0.005 ± 0.009	0.005 ± 0.009	0.005 ± 0.009	0.004 ± 0.004	0.004 ± 0.004	0.96 (0.55 to 1.36)	0.33 (-0.04 to 0.7)	0.021
XL_HDL_TG (mmol l <sup>-1</sup> )	0.009 ± 0.007	0.01 ± 0.007	0.009 ± 0.007	0.009 ± 0.005	0.008 ± 0.004	0.93 (0.68 to 1.18)	0.26 (-0.01 to 0.53)	<0.001
<b>Sevoflurane</b>								
Ala (mmol l <sup>-1</sup> )	0.28 ± 0.06	0.29 ± 0.06	0.27 ± 0.06	0.28 ± 0.04	0.25 ± 0.05	0.60 (0.4 to 0.81)	0.31 (0.06 to 0.57)	<0.001
<b>S-ketamine</b>								
Lac (mmol l <sup>-1</sup> )	0.65 ± 0.23	0.80 ± 0.34	0.82 ± 0.44	0.59 ± 0.2	0.53 ± 0.12	1.13 (0.65 to 1.6)	0.99 (0.47 to 1.51)	0.019
Glc (mmol l <sup>-1</sup> )	4.4 ± 0.53	4.71 ± 0.74	4.83 ± 0.81	4.14 ± 0.25	4.08 ± 0.22	0.85 (0.19 to 1.51)	1.21 (0.68 to 1.75)	0.042
Leu (mmol l <sup>-1</sup> )	0.069 ± 0.013	0.062 ± 0.013	0.062 ± 0.013	0.066 ± 0.008	0.07 ± 0.01	-0.64 (-0.92 to -0.36)	-0.89 (-1.27 to -0.52)	0.047

Data are given as mean ± SD or difference (95% CI). Timepoint 1 refers to the samples drawn before anaesthetic administration at baseline, timepoint 2 samples were drawn after 60-min anaesthetic administration and timepoint three samples were drawn 70 min after the cessation of anaesthetic administration. Changes between timepoints 1 vs. 2 and 1 vs. 3 are in SDS units, P values are shown for statistically significant changes. Before the calculation of the SDS, logarithmic transformation for skewed variables was performed, all metabolites shown here were logarithmically transformed before calculation of SDS. AcAce, acetoacetate; Ala, alanine; bOHBut, 3-hydroxybutyrate; Glc, glucose; Lac, lactate; Leu, leucine; SFA\_FA, ratio of saturated fatty acids to total fatty acids; XL\_HDL\_TG, triglycerides in very large HDL particles; XL\_VLDL\_FC, free cholesterol in very large VLDL particles.

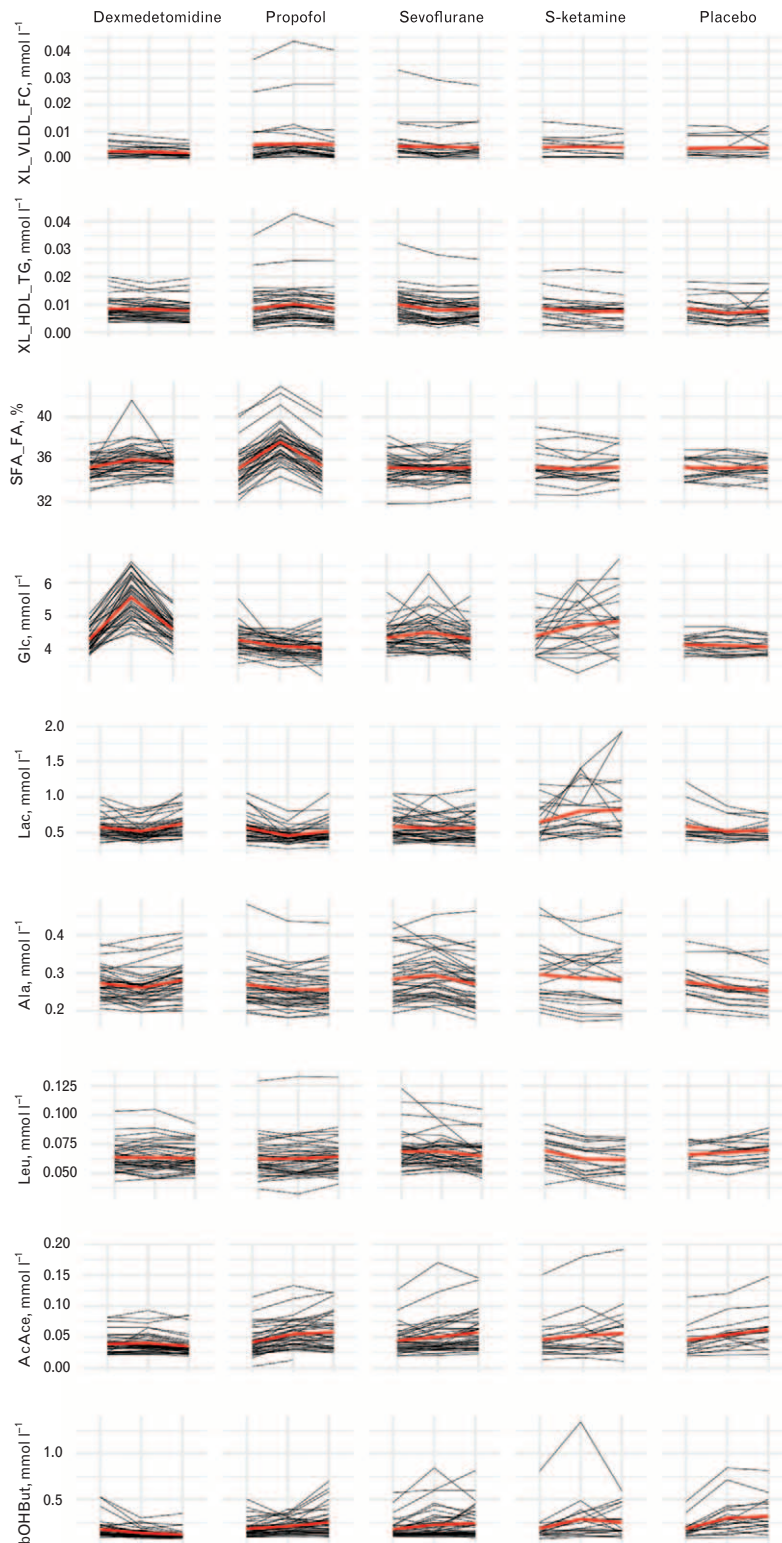
comparison, ingestion of a poly-unsaturated fatty acid rich meal in healthy individuals, lead to 75% increase in total triglycerides within 2 h.<sup>35</sup>

The in vitro analysis showed that the concentration of VLDL markers in the propofol group in vivo corresponded to VLDL values observed at 10-fold higher propofol concentration in the in vitro analysis (Appendix B, <http://links.lww.com/EJA/A604>). This, and further analysis of the NMR difference spectra suggested that accumulation of soybean oil, a propofol emulsion constituent, affected the observed change from chylomicrons and extremely large VLDL to medium VLDL markers and creatinine; other markers were largely unaffected. Accumulation of propofol emulsion constituents during a relatively brief 60-min infusion is highly interesting, as many of the known complications of propofol infusion are associated with derangements in lipid metabolism. Research has shown that propofol emulsion and Intralipid, a proxy for the lipid emulsion of propofol, caused similar changes in a small subset of blood lipids when the soybean oil content was equal between the groups.<sup>36</sup>

Somewhat surprisingly, the ratio of saturated fatty acids to total fatty acids (SFA\_FA) was increased in the propofol group. This is interesting, as soybean oil and Intralipid contain mostly unsaturated fatty acids. The unsaturated fatty acids decreased and saturated fatty acids increased, and were relatively unaffected by addition of propofol (1.75 to 17.5 µg l<sup>-1</sup>) in in vitro analysis (Appendix B, <http://links.lww.com/EJA/A604>). Previous in vitro studies on human skeletal and heart muscle cell bioenergetics have shown a profound inhibitory effect of propofol on fatty acid oxidation (FAO).<sup>37,38</sup> On the basis of previous research, after an overnight fast, FAO may account for up to 70% of total body energy expenditure.<sup>39</sup> In theory, a disruption of fatty acid utilisation could lead to changes in the circulating lipid profile. However, as free fatty acid availability exceeds the needs for FAO by a substantial amount, one could argue that changes in fatty acid ratios would take time to emerge. Whether propofol induced changes in fatty acid utilisation or FAO could affect the elevation of SFA\_FA remains unknown.

On the basis of our results, the lipid load induced by propofol could be reflected by the increase of SFA\_FA during a 60-min propofol infusion, whereas triglyceride levels remained relatively unchanged. It is worth contemplating whether the cumulation of propofol emulsion constituents or the alterations observed in the lipid profile could, in prolonged infusions and with vulnerable patients, be associated with the known complications of propofol infusion: hypertriglyceridaemia, hypertriglyceridaemia associated pancreatitis and the propofol infusion syndrome.<sup>19,20,40</sup> Currently, triglyceride levels of ICU patients are routinely screened for early diagnosis of propofol-induced hypertriglyceridaemia, which tends to develop after a median of 4 to 7 days in vulnerable

**Fig. 5** Line graphs showing the concentration changes in nine selected metabolites in individual subjects in each study group for three timepoints



First measurement at baseline before anaesthetic administration, second after 60 min of anaesthetic administration and third 70 min after the cessation of anaesthetic administration. The timepoint means are depicted with red lines. Ala, alanine; AcAce, acetoacetate; bOHBut, 3-hydroxybutyrate; Glc, glucose; Lac, lactate; Leu, leucine; SFA\_FA, ratio of saturated fatty acids to total fatty acids; XL\_HDL\_TG, triglycerides in very large HDL particles; XL\_VLDL\_FC, free cholesterol in very large VLDL particles. Line graphs of all metabolites are shown in Appendix A, <http://links.lww.com/EJA/A603>.

patients.<sup>19</sup> Whether changes in SFA\_FA levels precede the rise in circulating triglycerides remains an open question.

S-ketamine increased glucose and lactate. Lactate concentration was 53.8% higher than the corresponding concentration in placebo group at timepoint 2. This increase in lactate seems biologically significant, as the concentration of lactate at timepoint 2 in the S-ketamine group was 53 and 10.6% of the lactate levels observed during moderate and high-intensity exercise, respectively.<sup>41</sup> A hyperadrenergic state caused by S-ketamine offers a possible explanation for the observed increase in lactate and glucose.<sup>17,18,42,43</sup> The gradual decrease in tyrosine may also be explained by the hyperadrenergic state induced by S-ketamine, as tyrosine is funnelled to noradrenaline synthesis.

Interestingly, previous studies have shown that subanaesthetic doses of racemic ketamine increased cerebral blood flow (CBF) and regionally elevated cerebral glucose metabolic rate (GMR) without affecting the cerebral metabolic rate of oxygen (CMRO<sub>2</sub>). Anaesthetic doses of S-ketamine increased GMR in the thalamus, with only minor alterations in the CMRO<sub>2</sub>.<sup>26,44,45</sup> Increased GMR in the absence of increased CMRO<sub>2</sub> suggested a role of nonoxidative glucose metabolism in the CNS during ketamine infusion, possibly related to a ketamine-induced increase in glutamate cycling.<sup>45,46</sup> However, on the basis of current findings, it is not possible to identify the origins of the observed lactate release. The moderate increase in circulating lactate during isolated S-ketamine administration is nevertheless an interesting finding. Whether S-ketamine-induced lactataemia exaggerates lactataemia during critical illness in comparison to other anaesthetics is worth investigating.

S-ketamine decreased the levels of branched chain amino acids (BCAA) isoleucine, leucine and valine. The decrease in the aromatic amino acid (AAA) tyrosine reached statistical significance after the cessation of anaesthetic administration. At timepoint 3, the concentrations of isoleucine, leucine, valine and tyrosine were 8, 12.1, 2.5 and 7.4% lower in the S-ketamine group as compared with the placebo group. To offer a biological framework for these changes, a 35-min running exercise in healthy individuals decreased isoleucine by 9.0%, leucine 10.3%, valine 6.7% and tyrosine 4%, and the decrease in tyrosine was nonsignificant.<sup>47</sup> In this respect, the magnitude of changes in BCAA may have biological significance. It seems tempting to consider that reduction of BCAA could be linked to increased muscle activity. However, the energy demands of working skeletal muscle during a 35-min running exercise probably outweigh an adrenergic stimulus caused by S-ketamine at rest. Thus, the changes in muscle cell bioenergetics in response to adrenergic stimuli at rest seem insufficient to account for the observed decrease in BCAA levels.

Changes in BCAA concentrations are especially interesting, as S-ketamine has been approved for the treatment of “treatment-resistant” depression. BCAAs decrease brain uptake of AAAs due to a shared competitive transport mechanism across the blood-brain barrier. AAA tryptophan is a precursor of serotonin while AAA tyrosine, via transformation to dopamine, serves as a precursor for noradrenaline synthesis. Increases in circulating BCAAs lead to decreases in serotonin and catecholamine synthesis and release in the brain.<sup>48</sup> Thus, decreases in circulating BCAAs could, in theory, increase the effects of serotonin and noradrenaline in the brain, providing a plausible mechanism for the antidepressant effects of S-ketamine.<sup>39</sup> It is noteworthy, that the mechanism of action of commonly used antidepressants rely on selective serotonin and serotonin-noradrenaline reuptake inhibition. In addition, an earlier study revealed that the reduction in symptoms of major depressive disorder induced by sertraline was associated with decreases in BCAAs valine, leucine and isoleucine in serum samples.<sup>49</sup> Therefore, the current results may help explain the underlying mechanisms of the antidepressant effects of S-ketamine.<sup>39</sup>

On the basis of the current results, the most inert anaesthetic seems to be sevoflurane. Observed increases in motor restlessness may explain the increase in alanine concentration.<sup>47,50,51</sup> Sevoflurane sedation in ICU has been suggested as a viable alternative to propofol or midazolam sedation.<sup>52</sup> The current findings on the relative inertness of sevoflurane during short-term administration is a promising finding, which could prove useful in vulnerable patients with critical illness. However, the effects of prolonged sevoflurane sedation on the human metabolome remain unknown.

A few limitations must be addressed. First, the doses were moderate and the duration of anaesthetic exposure relatively short when compared with clinical anaesthesia. Second, only healthy male ASA I volunteers were studied, without the accompanying medications or comorbidities generally present with general anaesthesia. However, our aim was to discover and compare the direct effects of anaesthetic agents on the metabolic profile without the confounding factors usually present during the peri-operative period. This compromise leads to a relatively pure and comparable depiction of metabolic response to specific anaesthetics, yet limits our ability to offer clinically relevant predictions based on metabolite change. Further pragmatic studies are needed to assess the predictive value of metabolic changes associated with specific anaesthetics. Third, on the basis of the current results, we are unable to differentiate the effects of propofol from those of the accompanying lipid emulsion, as there was no control group for isolated lipid emulsion. However, as propofol is exclusively administered in lipid emulsion, the combined metabolic effects are of interest. Lastly, the metabolites that are closely related and share metabolic routes, are to some extent cross-correlated; this

has been illustrated in the heatmap in Appendix C, <http://links.lww.com/EJA/A605>.

In conclusion, this exploratory study revealed that, in absence of peri-operative confounding factors, a 1-h exposure to moderate doses of four routinely used anaesthetics induced unique alterations in the metabolic profile in healthy individuals as measured by NMR spectroscopy. Dexmedetomidine induced metabolic changes mirroring  $\alpha_2$ -adrenoceptor agonism and the inhibition of insulin secretion. Propofol emulsion influenced the lipid profile and elevated slightly the inflammatory marker GlycA. The inertness of sevoflurane might prove useful in vulnerable patients. Changes in amino acid profiles could be linked to the suggested antidepressant properties of S-ketamine. A need for further pragmatic studies on the clinical impact of anaesthetic induced shifts in the metabolome exists.

### Acknowledgements relating to this article

Assistance with the study: the authors thank the radiographers, anaesthesia nurses and laboratory nurses at Turku PET Centre for excellent technical assistance.

Financial support and sponsorship: this work was supported by Academy of Finland, Helsinki, Finland (grant numbers 266467, 266434); Jane and Aatos Erkkö Foundation, Helsinki, Finland; The Finnish Medical Foundation, Helsinki, Finland (JWL); The Emil Aaltonen Foundation, Tampere, Finland (JWL); and The Paulo Foundation, Espoo, Finland (LTL) and Orion Research Foundation, Espoo, Finland (LTL); Signe and Ane Gyllenberg Foundation, Helsinki, Finland (KJV); The Finnish Medical Foundation, Eero Matti Raninen Fund, Helsinki, Finland (AJN) and University of Turku, Finland (AJN).

Conflicts of interest: JWL has received a lecture compensation from Orion Pharma HTK and VA are employed by and hold stock options for Nightingale Health, a company offering NMR-based metabolic profiling. The rest of the authors have no conflicts of interest.

Presentation: none.

### References

- Guo L, Milburn MV, Ryals JA, *et al*. Plasma metabolomic profiles enhance precision medicine for volunteers of normal health. *Proc Natl Acad Sci U S A* 2015; **112**:E4901–E4910.
- Kaddurah-Daouk R, Weinshilboum R. Metabolomic signatures for drug response phenotypes: pharmacometabolomics enables precision medicine. *Clin Pharmacol Ther* 2015; **98**:71–75.
- Wurtz P, Raiko JR, Magnussen CG, *et al*. High-throughput quantification of circulating metabolites improves prediction of subclinical atherosclerosis. *Eur Heart J* 2012; **33**:2307–2316.
- Würtz P, Kangas AJ, Soininen P, *et al*. Quantitative serum nuclear magnetic resonance metabolomics in large-scale epidemiology: a primer on -omic technologies. *Am J Epidemiol* 2017; **186**:1084–1096.
- Rankin NJ, Preiss D, Welsh P, Sattar N. Applying metabolomics to cardiometabolic intervention studies and trials: past experiences and a roadmap for the future: Table 1. *Int J Epidemiol* 2016; **45**:1351–1371.
- Sliz E, Kettunen J, Holmes MV, *et al*. Metabolomic consequences of genetic inhibition of PCSK9 compared with statin treatment. *Circulation* 2018; **138**:2499–2512.
- Würtz P, Wang Q, Soininen P, *et al*. Metabolomic profiling of statin use and genetic inhibition of HMG-CoA reductase. *J Am Coll Cardiol* 2016; **67**:1200–1210.
- Wang Q, Wurtz P, Auro K, *et al*. Effects of hormonal contraception on systemic metabolism: cross-sectional and longitudinal evidence. *Int J Epidemiol* 2016; **45**:1445–1457.
- Rotroff DM, Corum DG, Motsinger-Reif A, *et al*. Metabolomic signatures of drug response phenotypes for ketamine and esketamine in subjects with refractory major depressive disorder: new mechanistic insights for rapid acting antidepressants. *Transl Psychiatry* 2016; **6**:e894.
- Ghini V, Unger FT, Tenori L, *et al*. Metabolomics profiling of preand postanesthesia plasma samples of colorectal patients obtained via Ficoll separation. *Metabolomics* 2015; **11**:1769–1778.
- Lambert DG. Omics and anaesthesia: pharmacogenomics, proteomics and metabolomics. *Anaesth Intensive Care* 2016; **17**:209–213.
- Lacoumenta S, Walsh ES, Waterman AE, *et al*. Effects of ketamine anaesthesia on the metabolic response to pelvic surgery. *Br J Anaesth* 1984; **56**:493–497.
- Disin-Oliveira RJ. Metabolism and metabolomics of ketamine: a toxicological approach. *Forensic Sci Res* 2017; **2**:2–10.
- Disin-Oliveira RJ. Metabolic profiles of propofol and fospropofol: clinical and forensic interpretative aspects. *Biomed Res Int* 2018; **2018**:6852857.
- Ghimire LV, Muszkat M, Sofowora GG, *et al*. Variation in the (2A) adrenoceptor gene and the effect of dexmedetomidine on plasma insulin and glucose. *Pharmacogenet Genomics* 2013; **23**:479–486.
- Peterhoff M, Sieg A, Brede M, *et al*. Inhibition of insulin secretion via distinct signaling pathways in alpha2-adrenoceptor knockout mice. *Eur J Endocrinol* 2003; **149**:343–350.
- Kohrs R, Durieux ME. Ketamine: teaching an old drug new tricks. *Anesth Analg* 1998; **87**:1186–1193.
- Baraka A, Harrison T, Kachachi T. Catecholamine levels after ketamine anaesthesia in man. *Anesth Analg* 1973; **52**:198–200.
- Devaud JC, Berger MM, Pannatier A, *et al*. Hypertriglyceridemia: a potential side effect of propofol sedation in critical illness. *Intensive Care Med* 2012; **38**:1990–1998.
- Devlin JW, Lau AK, Tanios MA. Propofol-associated hypertriglyceridemia and pancreatitis in the intensive care unit: an analysis of frequency and risk factors. *Pharmacotherapy* 2005; **25**:1348–1352.
- Krajčová A, Waldauf P, Anděl M, Duška F. Propofol infusion syndrome: a structured review of experimental studies and 153 published case reports. *Crit Care* 2015; **19**:398.
- Laaksonen L, Kallioinen M, Långsjö J, *et al*. Comparative effects of dexmedetomidine, propofol, sevoflurane, and S-ketamine on regional cerebral glucose metabolism in humans: a positron emission tomography study. *Br J Anaesth* 2018; **121**:281–290.
- Domino EF, Domino SE, Smith RE, *et al*. Ketamine kinetics in unmedicated and diazepam-premedicated subjects. *Clin Pharmacol Ther* 1984; **36**:645–653.
- Talke P, Lobo E, Brown R. Systemically administered alpha2-agonist-induced peripheral vasoconstriction in humans. *Anesthesiology* 2003; **99**:65–70.
- Marsh B, White M, Morton N, Kenny GN. Pharmacokinetic model driven infusion of propofol in children. *Br J Anaesth* 1991; **67**:41–48.
- Långsjö JW, Maksimow A, Salmi E, *et al*. S-ketamine anaesthesia increases cerebral blood flow in excess of the metabolic needs in humans. *Anesthesiology* 2005; **103**:258–268.
- Kaskinoro K, Maksimow A, Långsjö J, *et al*. Wide inter-individual variability of bispectral index and spectral entropy at loss of consciousness during increasing concentrations of dexmedetomidine, propofol, and sevoflurane. *Br J Anaesth* 2011; **107**:573–580.
- Kaskinoro K, Revonsuo A, Langsjö JW, *et al*. Returning from oblivion: imaging the neural core of consciousness. *J Neurosci* 2012; **32**:4935–4943.
- Soininen P, Kangas AJ, Wurtz P, *et al*. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. *Circ Cardiovasc Genet* 2015; **8**:192–206.
- Ren S, Hinzman AA, Kang EL, *et al*. Computational and statistical analysis of metabolomics data. *Metabolomics* 2015; **11**:1492–1513.
- Leren P. Comparison of effects on lipid metabolism of antihypertensive drugs with alpha- and beta-adrenergic antagonist properties. *Am J Med* 1987; **82**:31–35.
- Fischer K, Kettunen J, Wurtz P, *et al*. Biomarker profiling by nuclear magnetic resonance spectroscopy for the prediction of all-cause mortality: an observational study of 17,345 persons. *PLoS Med* 2014; **11**:e1001606.
- Ritchie SC, Wurtz P, Nath AP, *et al*. The biomarker GlycA is associated with chronic inflammation and predicts long-term risk of severe infection. *Cell Syst* 2015; **1**:293–301.
- Jaurila H, Koivukangas V, Koskela M, *et al*. 1H NMR based metabolomics in human sepsis and healthy serum. *Metabolites* 2020; **10**:70.
- Oyri LKL, Hansson P, Bogsrud MP, *et al*. Delayed postprandial TAG peak after intake of SFA compared with PUFA in subjects with and without familial hypercholesterolemia: a randomised controlled trial. *Br J Nutr* 2018; **119**:1142–1150.

- 36 De Sommer MR, Driessen JH, Willems CM, Lust PC. A comparative study on the effects of propofol in emulsion and Intralipid on fat metabolism. *Acta Anaesthesiol Belg* 1990; **41**:133–138.
- 37 Krajčová A, Løvsletten NG, Waldauf P, *et al.* Effects of propofol on cellular bioenergetics in human skeletal muscle cells. *Crit Care Med* 2017; **46**: e206–e212.
- 38 Urban T, Waldauf P, Krajčová A, *et al.* Kinetic characteristics of propofol-induced inhibition of electron-transfer chain and fatty acid oxidation in human and rodent skeletal and cardiac muscles. Bai Y, editor. *PLoS One* 2019; **14**:e0217254.
- 39 Coppack SW, Jensen MD, Miles JM. In vivo regulation of lipolysis in humans. *J Lipid Res* 1994; **177**–193.
- 40 Wolf A, Weir P, Segar P, *et al.* Impaired fatty acid oxidation in propofol infusion syndrome. *Lancet* 2001; **357**:606–607.
- 41 Peake JM, Tan SJ, Markworth JF, *et al.* Metabolic and hormonal responses to isoenergetic high-intensity interval exercise and continuous moderate-intensity exercise. *Am J Physiol Endocrinol Metab* 2014; **307**:E539–E552.
- 42 Qvisth V, Hagström-Toft E, Enoksson S, Bolinder J. Catecholamine regulation of local lactate production in vivo in skeletal muscle and adipose tissue: role of  $\beta$ -adrenoreceptor subtypes. *J Clin Endocrinol Metab* 2008; **93**:240–246.
- 43 Barth E, Albuszies G, Baumgart K, *et al.* Glucose metabolism and catecholamines. *Crit Care Med* 2007; **35**:S508–S518.
- 44 Långsjö JW, Kaisti KK, Aalto S, *et al.* Effects of subanesthetic doses of ketamine on regional cerebral blood flow, oxygen consumption, and blood volume in humans. *Anesthesiology* 2003; **99**:614–623.
- 45 Långsjö JW, Salmi E, Kaisti KK, *et al.* Effects of subanesthetic ketamine on regional cerebral glucose metabolism in humans. *Anesthesiology* 2004; **100**:1065–1071.
- 46 Hirota K, Lambert DG. Ketamine depression. *Br J Anaesth* 2018; **1198**–1202.
- 47 Pitkanen H, Mero A, Oja SS, *et al.* Serum amino acid responses to three different exercise sessions in male power athletes. *J Sports Med Phys Fitness* 2002; **42**:472–480.
- 48 Fernstrom JD. Branched-chain amino acids and brain function. *J Nutr* 2005; **135**:1539S–1546S.
- 49 Kaddurah-Daouk R, Bogdanov MB, Wikoff WR, *et al.* Pharmacometabolomic mapping of early biochemical changes induced by sertraline and placebo. *Transl Psychiatry* 2013; **3**:e223–e1223.
- 50 Kunst G, Graf BM, Schreiner R, *et al.* Differential effects of sevoflurane, isoflurane, and halothane on  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum of skeletal muscle. *Anesthesiology* 1999; **91**:179–186.
- 51 Felig P, Wahren J. Amino acid metabolism in exercising man. *J Clin invest* 1971; **50**:2703–2714.
- 52 Mesnil M, Capdevila X, Bringuier S, *et al.* Long-term sedation in intensive care unit: a randomized comparison between inhaled sevoflurane and intravenous propofol or midazolam. *Intensive Care Med* 2011; **37**:933–941.