Marley, C.J., Hodson, D., Brugniaux, J.V., Fall, L. and Bailey, D.M., 2017. Post-prandial hyperlipidaemia results in systemic nitrosative stress and impaired cerebrovascular function in the aged. *Clinical Science*, p.CS20171406.

Post-prandial hyperlipidaemia results in systemic nitrosative stress and impaired cerebrovascular function in the aged

Christopher J. Marley, PhD[1]; Danielle Hodson, PhD[1,2]; Julien V. Brugniaux[1,3], PhD; Lewis Fall, PhD[1] and Damian M. Bailey, PhD, FPVRI, FRSC, FACSM, FPS[1]

[1]Neurovascular Research Laboratory, Faculty of Life Sciences and Education, University of South Wales, UK; [2]School of Social and Health Sciences, Leeds Trinity University, UK and [3]School of Science and Health, Western Sydney University, Australia

Correspondence:
Professor Damian M. Bailey, Neurovascular Research Laboratory, Faculty of Life Sciences and Education, University of South Wales, UK, CF37 4AT.

Tel: +44 (0)1443 652296
Fax: +44 (0)1443 652285
e-mail: damian.bailey@southwales.ac.uk

Running Title: Post-prandial oxidative-nitrosative stress and cerebrovascular dysfunction

Key words: Aging, Cerebrovascular Function, High-Fat Meal, Hyperlipidaemia, Oxidative-Nitrosative Stress.

Abstract

Post-prandial hyperlipidaemia (PPH) acutely impairs systemic vascular endothelial function, potentially attributable to a free radical-mediated reduction in vascular nitric oxide (NO) bioavailability (oxidative-nitrosative stress). However, it remains to be determined whether this extends to the cerebrovasculature. To examine this, 38 (19 young (≤ 35 years) and 19 aged (≥ 60 years)) healthy males were recruited. Cerebrovascular function (middle cerebral artery velocity, MCAv) and cerebrovascular reactivity to hypercapnea ($CVR_{CO2Hyper}$) and hypocapnea ($CVR_{CO2Hypo}$) were determined via trans-cranial Doppler ultrasound and capnography. Venous blood samples were obtained for the assessment of triglycerides (photometry), glucose (photometry), insulin (radio-immunoassay), ascorbate free radical ($A^{\bullet-}$, electron paramagnetic resonance spectroscopy) and nitrite (NO_2^-, ozone-based chemiluminescence) in the fasted state prior to and 4 hours following consumption of a standardised high-fat meal (1,362 kcal; 130g of fat). Circulating triglycerides, glucose and insulin increased in both groups following the high-fat meal ($P < 0.05$), with triglycerides increasing by 1.37 $\pm$ 1.09 mmol/L in the young and 1.54 $\pm$ 1.00 mmol/L in the aged ($P < 0.05$). This resulted in an increased systemic formation of free radicals in the young ($P < 0.05$) but not the aged ($P > 0.05$) and corresponding reduction in NO_2^- in both groups ($P < 0.05$). While the meal had no effect on MCAv in either age group, $CVR_{CO2Hyper}$ was selectively impaired in the aged ($P < 0.05$). These findings indicate that PPH causes acute cerebrovascular dysfunction in the aged subsequent to systemic nitrosative stress.

Abbreviations: $A^{\bullet-}$, ascorbate free radical; BP, blood pressure; BMI, body mass index; CVR_{CO2}, cerebrovascular reactivity to changes in CO_2; $CVR_{CO2Hyper}$, cerebrovascular reactivity in response to hypercapnea; $CVR_{CO2Hypo}$, cerebrovascular reactivity in response to hypocapnea; CVs, coefficient of variations; EPR, electron paramagnetic resonance; MAP, mean arterial pressure; MCAv, middle cerebral artery velocity; NO, nitric oxide; NO_2^-, nitrite; PET_{CO2}, partial pressure of end-tidal CO_2; PPH, post-prandial hyperlipidaemia.

Introduction

The consumption of a high-fat meal is characterised by a state of post-prandial hyperlipidaemia (PPH) that can last for as long as 8 hours (1). During this time course, PPH has been shown to acutely impair systemic vascular endothelial function (2-5), that may be related to a free radical-mediated reduction in the vascular bioavailability of nitric oxide (NO) referred to as oxidative-nitrosative stress (6). However, the extent to which PPH impacts the cerebral vessels and may contribute to stroke risk has yet to be investigated. The reactivity of the cerebral vessels in response to changes in carbon dioxide (CVR_{CO2}) may be particularly vulnerable to PPH, since it is considered a reflection of cerebrovascular endothelial function (7). This would be of concern since any impairment in CVR_{CO2} translates into an increased risk of stroke (8). To date, only one study has investigated the impact of PPH on the cerebrovasculature and reported that it had no effect on young adults (9). Whether this is the same in the more "vulnerable, redox-reactive" aged brain remains to be established. To examine this, we designed a study with young and aged adults to determine if PPH in the latter would be associated with more marked impairments in cerebrovascular function subsequent to an age-related increase in systemic oxidative-nitrosative stress.

Materials and Methods

Participants

Following ethical approval (#12/11/2013) and written informed consent, 19 young males (aged 25 ± 6 years with a body mass index (BMI) of 23 ± 4 kg.m^2) and 19 aged males (aged 67 ± 5 years, BMI of 27 ± 3 kg.m^2) were recruited. All participants were non-smokers, without a history of cardiovascular, cerebrovascular or respiratory disease. All participants were encouraged to follow a low nitrate/nitrite diet and abstain from any antioxidant supplements prior to and throughout the duration of the study with specific instructions to avoid fruits, salads and cured meats.

Experimental Design

Data collection began at 08:00 hours following a 12-hour overnight fast. Measurements of cerebrovascular function and blood samples were obtained from each participant prior to and 4 hours following consumption of a standardised high-fat meal. The 4-hour mark was chosen as this is when the triglyceride response is considered to be at its peak (1). All procedures followed were in accordance with institutional guidelines.

High-Fat Meal

The test meal comprised of 130g of fat, 48g of carbohydrate and 9.5g of protein, as previously described by Patsch and colleagues (1). The total energy content of the meal was 1,362 calories.

Experimental Procedures

Cerebrovascular Function

Middle cerebral artery velocity (MCAv) was determined using a 2MHz pulsed Doppler ultrasound system (Multi-Dop X4, DWL Elektroniche Systeme GmbH, Sipplingen, Germany). Systolic, diastolic and mean arterial blood pressure (MAP) were monitored using finger photoplethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam, The Netherlands), with pulse pressure calculated as systolic minus diastolic blood pressure. Changes in the partial pressure of end-tidal CO_2 (PET_{CO2}) were determined via capnography (ML 206, ADInstruments Ltd, Oxford, UK). Data were sampled continuously at 1kHz and stored for off-line analysis. Following 10 minutes breathing room air, CVR_{CO2} was assessed in response to hypercapnea ($CVR_{CO2Hyper}$) by breathing an inspirate of 5% CO_2 with 21% O_2 and balanced nitrogen for 3 minutes. Following a 5-minute recovery breathing room air, CVR_{CO2} was also assessed in response to hypocapnea ($CVR_{CO2Hypo}$) via 3 minutes of controlled hyperventilation (15 breaths.min^{-1}). Both $CVR_{CO2Hyper}$ and $CVR_{CO2Hypo}$ were calculated as the percentage change in MCAv from baseline per 1 mmHg change in PET_{CO2} recorded during the final 30 seconds (average taken) of the respective challenge when steady-state had been achieved:

$$CVR_{CO2}\ (\%.mmHg^{-1}) = 100 \times \frac{\text{MCAv (final)} - \text{MCAv (baseline)}}{\text{MCAv (baseline)}} / [PET_{CO2}\ (\text{final}) - PET_{CO2}\ (\text{baseline})]$$

From these data, we derived the CVR_{CO2} range as a useful indication of the cerebral circulation's combined ability to respond to differential changes in CO_2 (10). This was calculated as the sum of the fractional vasodilation and vasoconstriction incurred during the respective hypercapnea and hypocapnea challenges as described:

$$CVR_{CO2}\ \text{range}\ (\%.mmHg^{-1}) = CVR_{CO2Hyper}\ (\%.mmHg^{-1}) + CVR_{CO2Hypo}\ (\%.mmHg^{-1})$$

Blood Samples

Venous blood samples were drawn without stasis from a forearm antecubital vein via an 18-gauge cannula into vacutainers. Once obtained, samples were centrifuged at 600 *g* for 10 minutes and the plasma/serum snap frozen in liquid nitrogen and stored at -80°C prior to batch analysis.

Lipids, Glucose and Insulin

Triglycerides and glucose were assessed using photometry (Randox Daytona Plus, Randox, County Antrim, UK). Insulin was assessed via radio-immunoassay (Invitron Insulin Assay Kit (IV2-001), Invitron Ltd, UK). The intra and inter-assay coefficient of variations (CVs) were both < 5% for all measures.

Nitric Oxide Bioavailability

Plasma nitrite (NO_2^-) was determined using ozone-based chemiluminescence (OBC Model 280i, NOA, Sievers, Boulder, CO, USA) following reduction by sodium iodide in acetic acid. The intra and inter-assay CVs were both < 5%.

Oxidative Stress

The ascorbate free radical ($A^{\bullet-}$) was employed as a direct measure of global oxidative stress as previously described (11). Plasma was injected into a high-sensitivity multiple-bore sample cell (AquaX; Bruker Daltonics Inc., Billerica, MA, USA) housed within an electron paramagnetic resonance (EPR) spectrometer operating at X-band (9.87 GHz). The intra and inter-assay CVs were both < 5%.

Statistical Analysis

Following confirmation of distribution normality (Shapiro-*W*-Wilk tests), data were analysed using a 2-way repeated measures (pre-meal *vs.* post-meal and young *vs.* old) analysis of variance. Where interaction effects were identified, differences were located using *Bonferonni*-corrected paired samples *t*-tests and independent samples *t*-tests. Significance was established at $P < 0.05$ and data expressed as mean $\pm$ standard deviation.

Results

At baseline, the aged presented with lower MCAv and higher fasted triglycerides, glucose, insulin, systolic blood pressure, pulse pressure, $CVR_{CO2Hyper}$ and corresponding CVR_{CO2} range ($P < 0.05$ *vs.* young, Table and Figure). During PPH, circulating triglycerides, glucose and insulin increased in both the young (triglycerides: 0.85 ± 0.50 to 2.22 ± 1.51 mmol/L; glucose: 5.48 ± 0.63 to 5.80 ± 0.63 mmol/L; insulin: 39 ± 19 to 97 ± 64 pmol/L, all $P < 0.05$ *vs.* pre-meal) and aged (triglycerides: 1.47 ± 0.70 to 3.02 ± 1.62 mmol/L; glucose: 6.07 ± 0.51 to 6.70 ± 1.03 mmol/L; insulin: 54 ± 30 to 148 ± 91 pmol/L; all $P < 0.05$ *vs.* pre-meal, Figure Panels A, B, C). This was associated with a selective increase in $A^{\bullet-}$ in the young but not the aged ($P < 0.05$ *vs.* pre-meal, Figure Panel D), whereas NO_2^- was shown to decrease in both groups ($P < 0.05$ *vs.* pre-meal, Figure Panel E). PPH had no effect on systolic blood pressure, diastolic blood pressure, MAP or pulse pressure ($P > 0.05$ *vs.* pre-meal, Table), but was associated with a selective reduction in $CVR_{CO2Hyper}$ and corresponding CVR_{CO2} range in the aged ($P < 0.05$ *vs.* pre-meal, Figure Panel F and Table).

Discussion

The major finding of the present study is that PPH was shown to selectively impair CVR_{CO2} in the aged, but not in the young adults and this was associated with a systemic reduction in bioactive NO_2^- in the absence of any systemic increase in free radical formation. This has important clinical implications given that an increasing proportion of the world's population is consuming high-fat diets and thus will spend large proportions of the day in a PPH state.

To the best of our knowledge, this is the first study to investigate the effect of PPH on the human cerebrovasculature across the adult lifespan. Our findings are in agreement with those by Dunn and Walter (9) who reported that PPH had no effect on the brain in young adults. Our study has extended these findings demonstrating for the first time that the aged brain is more vulnerable to the adverse effects of PPH in the form of reduced CVR_{CO2}, independent of any baseline differences in BMI between age groups. Thus, it would appear that the acute effects of PPH are not simply confined to the systemic circulation (2-5), but can equally impact the local cerebral circulation. Intrestingly, baseline CVR_{CO2} was higher in the aged compared to the young, which is in agreement with recent reports (12). Nonetheless, the reductions in CVR_{CO2} observed post-prandially in the aged, combined with the global reduction in MCAv, may enhance stroke risk (8) and increase vulnerability to neurodegenerative diseases (13). It is therefore important that nutritional guidelines are adhered to, which promote a varied and nutrient dense diet that is low in fat (14).

We sought to provide a mechanistic basis to explain these findings through specific assessment of systemic oxidative-nitrosative stress given that vascular endothelial dysfunction at least in the systemic and pulmonary circulation, has traditionally been attributed to a free radical-mediated reduction in endothelium-derived vascular NO bioavailability (11, 15). The observed changes in plasma NO_2^- support the concept that reductions in cerebrovascular function following a high-fat meal were mediated by a reduction in NO bioavailability, at least in the

aged. However, this does not appear to be caused by an increase in oxidative stress, since no additional elevations in $A^{\bullet-}$ were observed post-prandially. It was interesting to note that the baseline $A^{\bullet-}$ to which the aged were chronically exposed (*i.e.* prior to ingestion of the high-fat meal) was identical in magnitude to the acute increase observed post-prandially in the young, placing the degree of oxidative stress into clear physiological context, perhaps reflecting an "upper-limit" of oxidation. Alternatively, the net loss of plasma NO_2^- from the peripheral circulation may equally reflect increased consumption and concomitant liberation of NO or dinitrogen trioxide catalysed by acidic disproportionation (16) in an attempt to "salvage" vascular endothelial function in the chronically oxidatively-stressed cerebrovasculature of the older adults to maintain function.

Similar impairments in vascular function independent of changes in oxidative stress, albeit using indirect biomarkers of free radical-mediated lipid peroxidation have previously been reported (17, 18), though the underlying mechanisms remain to be established. A possible explanation may relate to the length of time that the aged brain has been exposed to increased levels of oxidative-stress across the lifespan (*i.e.* much longer than the young). This "chronic" effect may subsequently "blunt" the sensitivity of the systemic and cerebral vasculature to NO, a concept that has not previously been interrogated in this setting to the best of our knowledge. Thus, when the aged are challenged with the "additional" oxidative-nitrosative stress imposed by the high-fat meal, the vasculature is unable to synthesise sufficient NO and/or respond sufficiently to maintain function, resulting in a decrease in CVR_{CO2}. What is apparent is that the reduction in NO_2^- and subsequent impairment in vascular function observed in the aged are unlikely to be explained by changes in vascular compliance, since there were no changes in systolic blood pressure, diastolic blood pressure or pulse pressure following consumption of the high-fat meal. Similiarly, they are unlikely to be explained by any differences in the metabolic responses to the meal, since the relative increase in triglycerides, glucose and insulin

were comparable between both young and aged. Though, it is likely that the slightly elevated levels of triglycerides and glucose observed at baseline in the aged contributed to their chronic levels of oxidative stress, given the additional free radical production associated with their metabolism in larger quantities (19). These findings further highlight that additional research is warranted to better understand the causes of the deterioration in vascular function following the consumption of a high-fat meal, particularly in the aged.

A potential limitation to our study relates to between group differences in baseline measures, an almost unavoidable consequence when recruiting participants at polar opposites of the ageing continuum. However, the primary focus of the study was to determine within-group responses to the high-fat meal, rather than focus on the between-group responses. Thus, reducing the importance of having to match groups for anthropometrics, as well metabolic and vascular function. Our study would have also benefited by including an additional measure of general vascular reactivity such as flow-mediated dilatation to help explain our findings, since it is in part NO-dependant (20), similar to CVR_{CO2} (7). However, given the established literature that demonstrates a reduction in general vasoreactivity following consumption of a high-fat meal (2-5), it is plausible to assume that similar changes occurred in the present study.

In conclusion, the present findings demonstrate that a single high-fat meal can impair cerebrovascular function in the old but not in the young mediated in part by a systemic reduction in vascular NO bioavailability that was independent of any “additional” elevation in oxidative stress or changes in vascular compliance. These findings have important implications given the established relationship between impaired CVR_{CO2} and increased stroke risk.

Acknowledgements

We appreciate the commitment of the participants. Technical support was provided by Mr. Gareth Dunseath.

Declarations of Interest

The authors declare that there are no competing interests associated with the manuscript.

Funding

Damian Bailey is a Royal Society Wolfson Research Fellow (#WM170007). Damian Bailey received a Fellowship Award for Christopher Marley from the JPR Williams Trust.

Author Contributions

Damian Bailey and Christopher Marley led the acquisition/analysis/interpretation of data and wrote the original paper. Danielle Hodson and Julien Brugniaux made substanstial contributions to data acquisition and gave final approval of the version to be submitted. Lewis Fall made substantial contributions to data analysis and gave final approval of the version to be submitted. Damian Bailey was the Principal Investigator, recipient of funding and critically revised the original paper for important intellectual content and gave final approval of the version to be submitted including any revisons thereof.

Clinical Perspectives

i. Post-prandial hyperlipidaemia has been shown to acutely impair systemic vascular endothelial function, potentially attributable to increased oxidative-nitrosative stress. It remains to be determined whether this extends to the cerebrovasculature.

ii. Our findings identified that post-prandial hyperlipidaemia impairs cerebrovascular function in the aged, but not in the young and this was associated with a systemic reduction in nitric oxide bioavailability in the absence of increased oxidative stress or changes in vascular compliance.

iii. These findings have important clinical consequences since an increasing proportion of the world's population is consuming high-fat diets and an impairment in cerebrovascular function is associated with increased stroke risk and vulnerability to neurodegenerative diseases.

References

1. Patsch JR, Karlin JB, Scott LW, Smith LC, Gotto AM, Jr. Inverse relationship between blood levels of high density lipoprotein subfraction 2 and magnitude of postprandial lipemia. Proceedings of the National Academy of Sciences of the United States of America. 1983;80(5):1449-53.

2. Marchesi S, Lupattelli G, Schillaci G, Pirro M, Siepi D, Roscini AR, et al. Impaired flow-mediated vasoactivity during post-prandial phase in young healthy men. Atherosclerosis. 2000;153(2):397-402.

3. Tsai WC, Li YH, Lin CC, Chao TH, Chen JH. Effects of oxidative stress on endothelial function after a high-fat meal. Clin Sci (Lond). 2004;106(3):315-9.

4. Vogel RA, Corretti MC, Plotnick GD. Effect of a single high-fat meal on endothelial function in healthy subjects. The American journal of cardiology. 1997;79(3):350-4.

5. West SG, Hecker KD, Mustad VA, Nicholson S, Schoemer SL, Wagner P, et al. Acute effects of monounsaturated fatty acids with and without omega-3 fatty acids on vascular reactivity in individuals with type 2 diabetes. Diabetologia. 2005;48(1):113-22.

6. Bailey DM, Evans KA, McEneny J, Young IS, Hullin DA, James PE, et al. Exercise-induced oxidative-nitrosative stress is associated with impaired dynamic cerebral autoregulation and blood-brain barrier leakage. Experimental physiology. 2011;96(11):1196-207.

7. Lavi S, Gaitini D, Milloul V, Jacob G. Impaired cerebral CO2 vasoreactivity: association with endothelial dysfunction. American journal of physiology Heart and circulatory physiology. 2006;291(4):H1856-61.

8. Gupta A, Chazen JL, Hartman M, Delgado D, Anumula N, Shao H, et al. Cerebrovascular reserve and stroke risk in patients with carotid stenosis or occlusion: a

systematic review and meta-analysis. Stroke; a journal of cerebral circulation. 2012;43(11):2884-91.

9. Dunn WG, Walters MR. A randomised crossover trial of the acute effects of a deep-fried Mars bar or porridge on the cerebral vasculature. Scott Med J. 2014;59(4):182-7.

10. Bailey DM, Jones DW, Sinnott A, Brugniaux JV, New KJ, Hodson D, et al. Impaired cerebral haemodynamic function associated with chronic traumatic brain injury in professional boxers. Clin Sci (Lond). 2013;124(3):177-89.

11. Bailey DM, Dehnert C, Luks AM, Menold E, Castell C, Schendler G, et al. High-altitude pulmonary hypertension is associated with a free radical-mediated reduction in pulmonary nitric oxide bioavailability. The Journal of physiology. 2010;588(Pt 23):4837-47.

12. Zhu YS, Tarumi T, Tseng BY, Palmer DM, Levine BD, Zhang R. Cerebral vasomotor reactivity during hypo- and hypercapnia in sedentary elderly and Masters athletes. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2013;33(8):1190-6.

13. Gorelick PB, Scuteri A, Black SE, Decarli C, Greenberg SM, Iadecola C, et al. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the american heart association/american stroke association. Stroke; a journal of cerebral circulation. 2011;42(9):2672-713.

14. Public Health England. A Quick Guide to the Government's Healthy Eating Recommendations 2017 [Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/595133/A_quick_guide_to_govt_healthy_eating.pdf.

15. Gryglewski RJ, Palmer RM, Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. Nature. 1986;320(6061):454-6.

16. Bailey DM, Rasmussen P, Overgaard M, Evans KA, Bohm AM, Seifert T, et al. Nitrite and S-Nitrosohemoglobin Exchange Across the Human Cerebral and Femoral Circulation: Relationship to Basal and Exercise Blood Flow Responses to Hypoxia. Circulation. 2017;135(2):166-76.

17. Gopaul NK, Zacharowski K, Halliwell B, Anggard EE. Evaluation of the postprandial effects of a fast-food meal on human plasma F(2)-isoprostane levels. Free Radic Biol Med. 2000;28(5):806-14.

18. Williams MJ, Sutherland WH, McCormick MP, de Jong SA, Walker RJ, Wilkins GT. Impaired endothelial function following a meal rich in used cooking fat. Journal of the American College of Cardiology. 1999;33(4):1050-5.

19. Roberts CK, Vaziri ND, Wang XQ, Barnard RJ. Enhanced NO inactivation and hypertension induced by a high-fat, refined-carbohydrate diet. Hypertension. 2000;36(3):423-9.

20. Green DJ, Dawson EA, Groenewoud HM, Jones H, Thijssen DH. Is flow-mediated dilation nitric oxide mediated?: A meta-analysis. Hypertension. 2014;63(2):376-82.

Table. Changes in cerebrovascular function following a high-fat meal.

Age Group	Young (*n* = 19)		Aged (*n* = 19)		*P* Values		
	Pre-Meal	Post-Meal	Pre-Meal	Post-Meal	Age	Meal	Age × Meal
MCAv ($cm.s^{-1}$)	62 ± 13	61 ± 10	48 ± 12	46 ± 10	**0.00**	0.37	0.67
Systolic BP (mmHg)	123 ± 10	122 ± 9	132 ± 16	129 ± 16	**0.04**	0.46	0.50
Diastolic BP (mmHg)	70 ± 10	68 ± 8	70 ± 11	68 ± 10	0.98	0.24	0.84
MAP (mmHg)	87 ± 9	86 ± 7	91 ± 11	89 ± 10	0.85	0.40	0.72
Pulse Pressure (mmHg)	52 ± 7	54 ± 9	62 ± 14	61 ± 13	**0.02**	0.69	0.28
$CVR_{CO2Hyper}$ ($\%.mmHg^{-1}$)	2.68 ± 0.85	2.39 ± 0.85	5.46 ± 1.71†	4.07 ± 1.06*†	**0.00**	**0.00**	**0.00**
$CVR_{CO2Hypo}$ ($\%.mmHg^{-1}$)	2.49 ± 0.45	2.12 ± 0.58	2.47 ± 0.89	2.28 ± 1.29	0.77	**0.05**	0.51
CVR_{CO2} range ($\%.mmHg^{-1}$)	5.17 ± 0.90	4.51 ± 1.16	8.21 ± 1.95†	6.35 ± 1.61*†	**0.00**	**0.00**	**0.01**

Values are mean ± SD; MCAv, middle cerebral artery velocity; BP, blood pressure; MAP, mean arterial blood pressure; $CVR_{CO2Hyper}$/$CVR_{CO2Hypo}$, cerebrovascular reactivity to hypercapnea/hypocapnea; CVR_{CO2} range, combination of $CVR_{CO2Hyper}$ and $CVR_{CO2Hypo}$. */† = difference within/between age groups ($P < 0.05$)

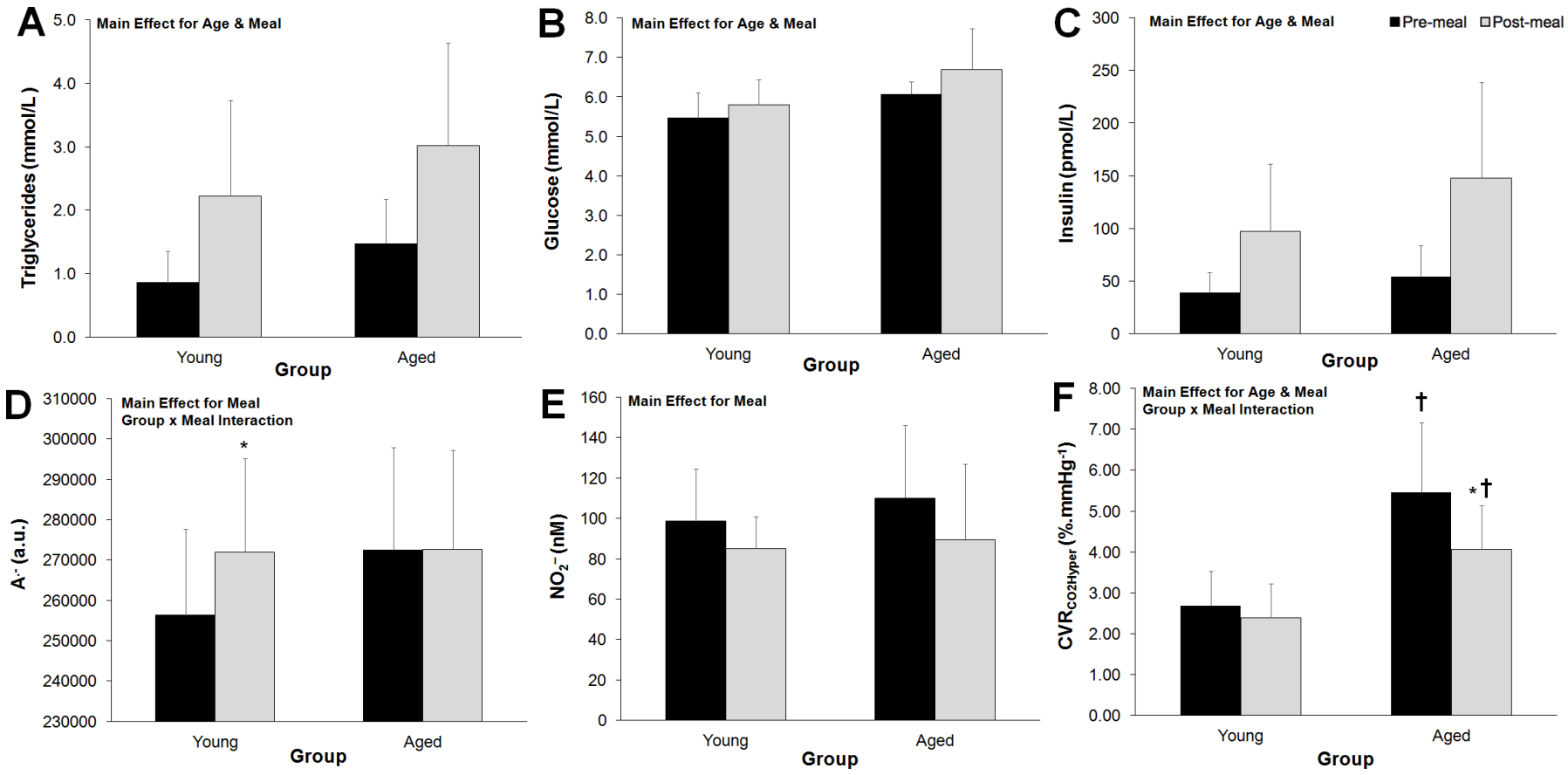

Figure. Metabolic (A-E) and haemodynamic (F) responses to a high-fat meal.

Data are mean ± SD; Young *n* = 19, Aged *n* = 19; *different *vs.* pre-meal for given Group ($P < 0.05$), †different *vs.* Group ($P < 0.05$).