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

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

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

**1 Abstract**

2 Post-prandial hyperlipidaemia (PPH) acutely impairs systemic vascular endothelial function,  
3 potentially attributable to a free radical-mediated reduction in vascular nitric oxide (NO)  
4 bioavailability (oxidative-nitrosative stress). However, it remains to be determined whether  
5 this extends to the cerebrovasculature. To examine this, 38 (19 young ( $\leq 35$  years) and 19 aged  
6 ( $\geq 60$  years)) healthy males were recruited. Cerebrovascular function (middle cerebral artery  
7 velocity, MCAv) and cerebrovascular reactivity to hypercapnea ( $\text{CVR}_{\text{CO}_2\text{Hyper}}$ ) and hypocapnea  
8 ( $\text{CVR}_{\text{CO}_2\text{Hypo}}$ ) were determined via trans-cranial Doppler ultrasound and capnography. Venous  
9 blood samples were obtained for the assessment of triglycerides (photometry), glucose  
10 (photometry), insulin (radio-immunoassay), ascorbate free radical ( $\text{A}^{\cdot-}$ , electron paramagnetic  
11 resonance spectroscopy) and nitrite ( $\text{NO}_2^-$ , ozone-based chemiluminescence) in the fasted state  
12 prior to and 4 hours following consumption of a standardised high-fat meal (1,362 kcal; 130g  
13 of fat). Circulating triglycerides, glucose and insulin increased in both groups following the  
14 high-fat meal ( $P < 0.05$ ), with triglycerides increasing by  $1.37 \pm 1.09$  mmol/L in the young and  
15  $1.54 \pm 1.00$  mmol/L in the aged ( $P < 0.05$ ). This resulted in an increased systemic formation  
16 of free radicals in the young ( $P < 0.05$ ) but not the aged ( $P > 0.05$ ) and corresponding reduction  
17 in  $\text{NO}_2^-$  in both groups ( $P < 0.05$ ). While the meal had no effect on MCAv in either age group,  
18  $\text{CVR}_{\text{CO}_2\text{Hyper}}$  was selectively impaired in the aged ( $P < 0.05$ ). These findings indicate that PPH  
19 causes acute cerebrovascular dysfunction in the aged subsequent to systemic nitrosative stress.

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25 **Abbreviations:**  $A^{\bullet-}$ , ascorbate free radical; BP, blood pressure; BMI, body mass index;  
26  $CVR_{CO_2}$ , cerebrovascular reactivity to changes in  $CO_2$ ;  $CVR_{CO_2Hyper}$ , cerebrovascular reactivity  
27 in response to hypercapnea;  $CVR_{CO_2Hypo}$ , cerebrovascular reactivity in response to hypocapnea;  
28 CVs, coefficient of variations; EPR, electron paramagnetic resonance; MAP, mean arterial  
29 pressure; MCAv, middle cerebral artery velocity; NO, nitric oxide;  $NO_2^-$ , nitrite;  $PET_{CO_2}$ ,  
30 partial pressure of end-tidal  $CO_2$ ; PPH, post-prandial hyperlipidaemia.

**31 Introduction**

32 The consumption of a high-fat meal is characterised by a state of post-prandial hyperlipidaemia  
33 (PPH) that can last for as long as 8 hours (1). During this time course, PPH has been shown to  
34 acutely impair systemic vascular endothelial function (2-5), that may be related to a free  
35 radical-mediated reduction in the vascular bioavailability of nitric oxide (NO) referred to as  
36 oxidative-nitrosative stress (6). However, the extent to which PPH impacts the cerebral vessels  
37 and may contribute to stroke risk has yet to be investigated. The reactivity of the cerebral  
38 vessels in response to changes in carbon dioxide ( $CVR_{CO_2}$ ) may be particularly vulnerable to  
39 PPH, since it is considered a reflection of cerebrovascular endothelial function (7). This would  
40 be of concern since any impairment in  $CVR_{CO_2}$  translates into an increased risk of stroke (8).  
41 To date, only one study has investigated the impact of PPH on the cerebrovasculature and  
42 reported that it had no effect on young adults (9). Whether this is the same in the more  
43 “vulnerable, redox-reactive” aged brain remains to be established. To examine this, we  
44 designed a study with young and aged adults to determine if PPH in the latter would be  
45 associated with more marked impairments in cerebrovascular function subsequent to an age-  
46 related increase in systemic oxidative-nitrosative stress.

47

## 48 **Materials and Methods**

### 49 *Participants*

50 Following ethical approval (#12/11/2013) and written informed consent, 19 young males (aged  
51  $25 \pm 6$  years with a body mass index (BMI) of  $23 \pm 4$  kg.m<sup>2</sup>) and 19 aged males (aged  $67 \pm 5$   
52 years, BMI of  $27 \pm 3$  kg.m<sup>2</sup>) were recruited. All participants were non-smokers, without a  
53 history of cardiovascular, cerebrovascular or respiratory disease. All participants were  
54 encouraged to follow a low nitrate/nitrite diet and abstain from any antioxidant supplements  
55 prior to and throughout the duration of the study with specific instructions to avoid fruits, salads  
56 and cured meats.

57

### 58 *Experimental Design*

59 Data collection began at 08:00 hours following a 12-hour overnight fast. Measurements of  
60 cerebrovascular function and blood samples were obtained from each participant prior to and  
61 4 hours following consumption of a standardised high-fat meal. The 4-hour mark was chosen  
62 as this is when the triglyceride response is considered to be at its peak (1). All procedures  
63 followed were in accordance with institutional guidelines.

64

### 65 *High-Fat Meal*

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

68

69

70



## 71 *Experimental Procedures*

### 72 *Cerebrovascular Function*

73 Middle cerebral artery velocity (MCAv) was determined using a 2MHz pulsed Doppler  
 74 ultrasound system (Multi-Dop X4, DWL Elektronische Systeme GmbH, Sipplingen, Germany).  
 75 Systolic, diastolic and mean arterial blood pressure (MAP) were monitored using finger  
 76 photoplethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam, The  
 77 Netherlands), with pulse pressure calculated as systolic minus diastolic blood pressure.  
 78 Changes in the partial pressure of end-tidal CO<sub>2</sub> (PET<sub>CO2</sub>) were determined via capnography  
 79 (ML 206, ADInstruments Ltd, Oxford, UK). Data were sampled continuously at 1kHz and  
 80 stored for off-line analysis. Following 10 minutes breathing room air, CVR<sub>CO2</sub> was assessed in  
 81 response to hypercapnea (CVR<sub>CO2Hyper</sub>) by breathing an inspirate of 5% CO<sub>2</sub> with 21% O<sub>2</sub> and  
 82 balanced nitrogen for 3 minutes. Following a 5-minute recovery breathing room air, CVR<sub>CO2</sub>  
 83 was also assessed in response to hypocapnea (CVR<sub>CO2Hypo</sub>) via 3 minutes of controlled  
 84 hyperventilation (15 breaths.min<sup>-1</sup>). Both CVR<sub>CO2Hyper</sub> and CVR<sub>CO2Hypo</sub> were calculated as the  
 85 percentage change in MCAv from baseline per 1 mmHg change in PET<sub>CO2</sub> recorded during the  
 86 final 30 seconds (average taken) of the respective challenge when steady-state had been  
 87 achieved:

88

$$89 \text{ CVR}_{\text{CO}_2} (\% \cdot \text{mmHg}^{-1}) = 100 \times \frac{\Delta \text{MCAv} (\%)}{\Delta \text{PET}_{\text{CO}_2} (\text{mmHg})} / [\text{PET}_{\text{CO}_2} (\text{final}) - \text{PET}_{\text{CO}_2} (\text{baseline})]$$

90

91 From these data, we derived the CVR<sub>CO2</sub> range as a useful indication of the cerebral  
 92 circulation's combined ability to respond to differential changes in CO<sub>2</sub> (10). This was  
 93 calculated as the sum of the fractional vasodilation and vasoconstriction incurred during the  
 94 respective hypercapnea and hypocapnea challenges as described:

95

$$96 \text{ CVR}_{\text{CO}_2} \text{ range } (\% \cdot \text{mmHg}^{-1}) = \text{CVR}_{\text{CO}_2\text{Hyper}} (\% \cdot \text{mmHg}^{-1}) + \text{CVR}_{\text{CO}_2\text{Hypo}} (\% \cdot \text{mmHg}^{-1})$$

97 ***Blood Samples***

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

102

103 ***Lipids, Glucose and Insulin***

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

108

109 ***Nitric Oxide Bioavailability***

110 Plasma nitrite ( $\text{NO}_2^-$ ) was determined using ozone-based chemiluminescence (OBC Model  
111 280i, NOA, Sievers, Boulder, CO, USA) following reduction by sodium iodide in acetic acid.  
112 The intra and inter-assay CVs were both < 5%.

113

114 ***Oxidative Stress***

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

120

121

**122    *Statistical Analysis***

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

128

**129 Results**

130 At baseline, the aged presented with lower MCAv and higher fasted triglycerides, glucose,  
131 insulin, systolic blood pressure, pulse pressure,  $CVR_{CO_2Hyper}$  and corresponding  $CVR_{CO_2}$  range  
132 ( $P < 0.05$  vs. young, Table and Figure). During PPH, circulating triglycerides, glucose and  
133 insulin increased in both the young (triglycerides:  $0.85 \pm 0.50$  to  $2.22 \pm 1.51$  mmol/L; glucose:  
134  $5.48 \pm 0.63$  to  $5.80 \pm 0.63$  mmol/L; insulin:  $39 \pm 19$  to  $97 \pm 64$  pmol/L, all  $P < 0.05$  vs. pre-  
135 meal) and aged (triglycerides:  $1.47 \pm 0.70$  to  $3.02 \pm 1.62$  mmol/L; glucose:  $6.07 \pm 0.51$  to  $6.70$   
136  $\pm 1.03$  mmol/L; insulin:  $54 \pm 30$  to  $148 \pm 91$  pmol/L; all  $P < 0.05$  vs. pre-meal, Figure Panels  
137 A, B, C). This was associated with a selective increase in  $A^+$  in the young but not the aged ( $P$   
138  $< 0.05$  vs. pre-meal, Figure Panel D), whereas  $NO_2^-$  was shown to decrease in both groups ( $P$   
139  $< 0.05$  vs. pre-meal, Figure Panel E). PPH had no effect on systolic blood pressure, diastolic  
140 blood pressure, MAP or pulse pressure ( $P > 0.05$  vs. pre-meal, Table), but was associated with  
141 a selective reduction in  $CVR_{CO_2Hyper}$  and corresponding  $CVR_{CO_2}$  range in the aged ( $P < 0.05$   
142 vs. pre-meal, Figure Panel F and Table).

**143 Discussion**

144 The major finding of the present study is that PPH was shown to selectively impair  $CVR_{CO_2}$  in  
145 the aged, but not in the young adults and this was associated with a systemic reduction in  
146 bioactive  $NO_2^-$  in the absence of any systemic increase in free radical formation. This has  
147 important clinical implications given that an increasing proportion of the world's population is  
148 consuming high-fat diets and thus will spend large proportions of the day in a PPH state.

149 To the best of our knowledge, this is the first study to investigate the effect of PPH on the  
150 human cerebrovasculature across the adult lifespan. Our findings are in agreement with those  
151 by Dunn and Walter (9) who reported that PPH had no effect on the brain in young adults. Our  
152 study has extended these findings demonstrating for the first time that the aged brain is more  
153 vulnerable to the adverse effects of PPH in the form of reduced  $CVR_{CO_2}$ , independent of any  
154 baseline differences in BMI between age groups. Thus, it would appear that the acute effects  
155 of PPH are not simply confined to the systemic circulation (2-5), but can equally impact the  
156 local cerebral circulation. Interestingly, baseline  $CVR_{CO_2}$  was higher in the aged compared to  
157 the young, which is in agreement with recent reports (12). Nonetheless, the reductions in  
158  $CVR_{CO_2}$  observed post-prandially in the aged, combined with the global reduction in  $MCA_v$ ,  
159 may enhance stroke risk (8) and increase vulnerability to neurodegenerative diseases (13). It is  
160 therefore important that nutritional guidelines are adhered to, which promote a varied and  
161 nutrient dense diet that is low in fat (14).

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

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

178 Similar impairments in vascular function independent of changes in oxidative stress, albeit  
179 using indirect biomarkers of free radical-mediated lipid peroxidation have previously been  
180 reported (17, 18), though the underlying mechanisms remain to be established. A possible  
181 explanation may relate to the length of time that the aged brain has been exposed to increased  
182 levels of oxidative-stress across the lifespan (*i.e.* much longer than the young). This “chronic”  
183 effect may subsequently “blunt” the sensitivity of the systemic and cerebral vasculature to NO,  
184 a concept that has not previously been interrogated in this setting to the best of our knowledge.  
185 Thus, when the aged are challenged with the “additional” oxidative-nitrosative stress imposed  
186 by the high-fat meal, the vasculature is unable to synthesise sufficient NO and/or respond  
187 sufficiently to maintain function, resulting in a decrease in  $CVR_{CO_2}$ . What is apparent is that  
188 the reduction in  $NO_2^-$  and subsequent impairment in vascular function observed in the aged are  
189 unlikely to be explained by changes in vascular compliance, since there were no changes in  
190 systolic blood pressure, diastolic blood pressure or pulse pressure following consumption of  
191 the high-fat meal. Similarly, they are unlikely to be explained by any differences in the  
192 metabolic responses to the meal, since the relative increase in triglycerides, glucose and insulin

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

199 A potential limitation to our study relates to between group differences in baseline measures,  
200 an almost unavoidable consequence when recruiting participants at polar opposites of the  
201 ageing continuum. However, the primary focus of the study was to determine within-group  
202 responses to the high-fat meal, rather than focus on the between-group responses. Thus,  
203 reducing the importance of having to match groups for anthropometrics, as well metabolic and  
204 vascular function. Our study would have also benefited by including an additional measure of  
205 general vascular reactivity such as flow-mediated dilatation to help explain our findings, since  
206 it is in part NO-dependant (20), similar to  $CVR_{CO_2}$  (7). However, given the established  
207 literature that demonstrates a reduction in general vasoreactivity following consumption of a  
208 high-fat meal (2-5), it is plausible to assume that similar changes occurred in the present study.

209 In conclusion, the present findings demonstrate that a single high-fat meal can impair  
210 cerebrovascular function in the old but not in the young mediated in part by a systemic  
211 reduction in vascular NO bioavailability that was independent of any “additional” elevation in  
212 oxidative stress or changes in vascular compliance. These findings have important implications  
213 given the established relationship between impaired  $CVR_{CO_2}$  and increased stroke risk.

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217

**218 Declarations of Interest**

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

220

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224

**225 Author Contributions**

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

233



**234 Clinical Perspectives**

- 235 i. Post-prandial hyperlipidaemia has been shown to acutely impair systemic vascular  
236 endothelial function, potentially attributable to increased oxidative-nitrosative stress. It  
237 remains to be determined whether this extends to the cerebrovasculature.
- 238 ii. Our findings identified that post-prandial hyperlipidaemia impairs cerebrovascular function  
239 in the aged, but not in the young and this was associated with a systemic reduction in nitric  
240 oxide bioavailability in the absence of increased oxidative stress or changes in vascular  
241 compliance.
- 242 iii. These findings have important clinical consequences since an increasing proportion of the  
243 world's population is consuming high-fat diets and an impairment in cerebrovascular  
244 function is associated with increased stroke risk and vulnerability to neurodegenerative  
245 diseases.

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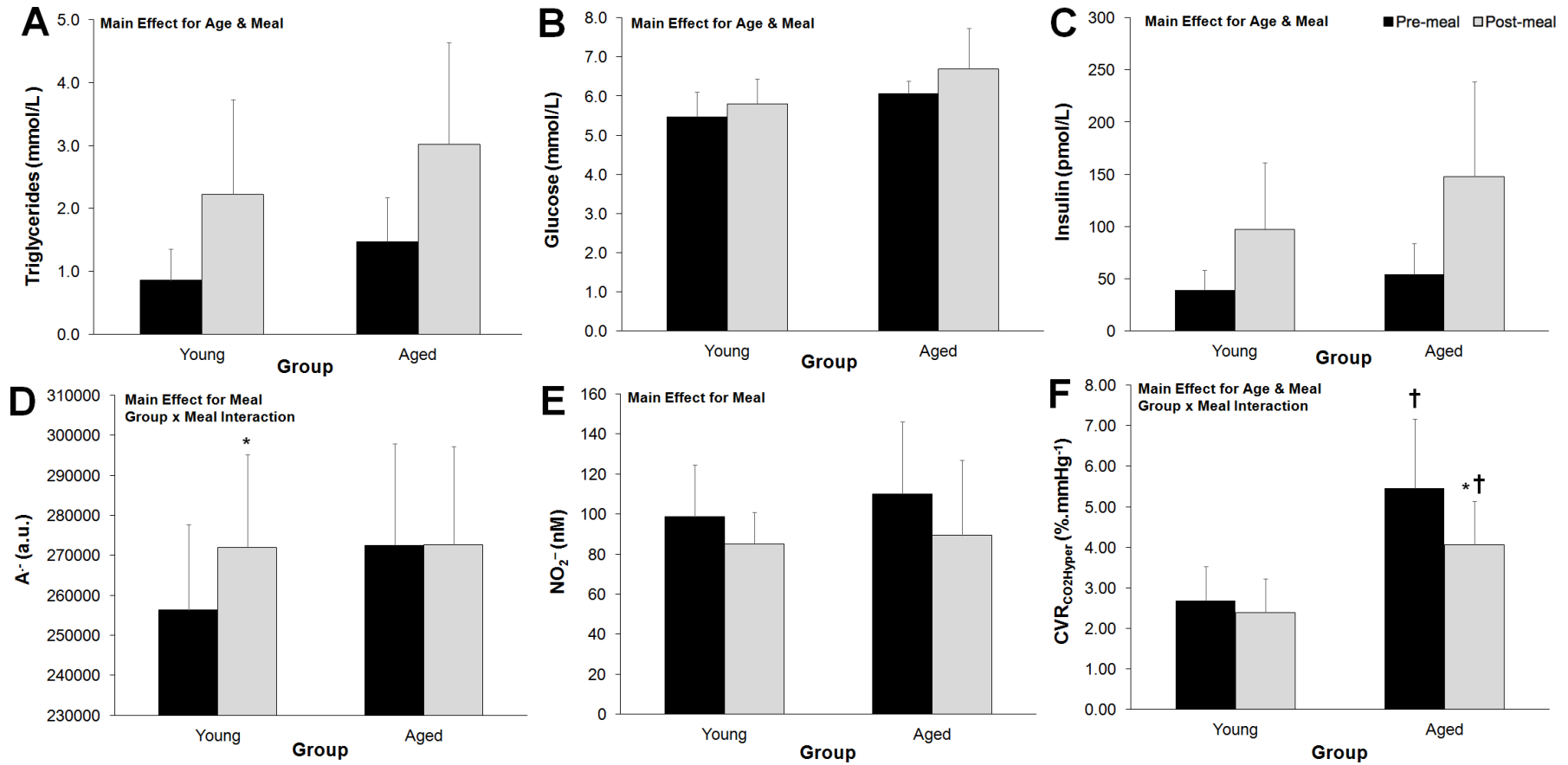
**Table.** Changes in cerebrovascular function following a high-fat meal.

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

Values are mean ± SD; MCAv, middle cerebral artery velocity; BP, blood pressure; MAP, mean arterial blood pressure;

CVR<sub>CO2Hyper</sub>/CVR<sub>CO2Hypo</sub>, cerebrovascular reactivity to hypercapnea/hypocapnea; CVR<sub>CO2</sub> range, combination of CVR<sub>CO2Hyper</sub> and

CVR<sub>CO2Hypo</sub>. \*/† = difference within/between age groups (*P* < 0.05)



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

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

24

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26