



Citation for published version:

Campbell, MD, Walker, M, Ajjan, RA, Birch, KM, Gonzalez, J & West, DJ 2017, 'An additional bolus of rapid-acting insulin to normalise postprandial cardiovascular risk factors following high-carbohydrate high-fat meal in patients with type 1 diabetes: A randomised controlled trial', *Diabetes and Vascular Disease Research*, vol. 14, no. 4, pp. 336-344. <https://doi.org/10.1177/1479164117698918>

DOI:

[10.1177/1479164117698918](https://doi.org/10.1177/1479164117698918)

Publication date:

2017

Document Version

Peer reviewed version

[Link to publication](#)

Campbell, M. D., Walker, M., Ajjan, R. A., Birch, K. M., Gonzalez, J., & West, D. J. (2017). An additional bolus of rapid-acting insulin to normalise postprandial cardiovascular risk factors following high-carbohydrate high-fat meal in patients with type 1 diabetes: A randomised controlled trial: Cardiovascular risk and high fat feeding in type 1 diabetes. *Diabetes and Vascular Disease Research*, 14(4), 336-344. Copyright © 2017 The Author(s). Reprinted by permission of SAGE Publications.

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1 **Title**

2 An additional bolus of rapid-acting insulin to normalise postprandial cardiovascular risk factors
3 following high-carbohydrate high-fat meal in patients with type 1 diabetes: A randomised
4 controlled trial

5 **Short title**

6 Cardiovascular risk and high fat feeding in type 1 diabetes

7

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25

26 **Abstract**

27 **Aim:** To evaluate an additional rapid-acting insulin bolus on postprandial lipaemia,
28 inflammation, and pro-coagulation following high-carbohydrate high-fat feeding in people
29 with type 1 diabetes.

30 **Methods:** Ten males with type 1 diabetes (HbA_{1c} 52.5 ± 5.9 mmol/mol [$7.0 \pm 0.5\%$]) underwent
31 three conditions: 1) a low-fat meal with normal bolus insulin (**LF**), 2), a high-fat meal with
32 normal bolus insulin (**HF**), 3) a high-fat meal with normal bolus insulin with an additional 30%
33 insulin bolus administered 3-hrs post-meal (**HFA**). Meals had identical carbohydrate and
34 protein content and bolus insulin dose determined by carbohydrate-counting. Blood was
35 sampled periodically for 6-hr post-meal and analysed for TG, NEFA, $APOB_{48}$, glucagon, TNF-
36 α , fibrinogen, HTF activity, and PAI-1. Continuous glucose monitoring captured interstitial
37 glucose responses.

38 **Results:** TG concentrations following **LF** remained similar to baseline, whereas TG levels
39 following **HF** were significantly greater throughout the 6-hour observation period. The
40 additional insulin bolus (**HFA**) normalised TG similarly to **LF** 3-6-hrs following the meal. **HF**
41 was associated with late postprandial elevations in TNF- α , whereas **LF** and **HFA** was not.
42 Fibrinogen, PAI-1, and TFP levels were similar between conditions.

43 **Conclusions:** Additional bolus insulin 3-hrs following a high-carbohydrate high-fat meal
44 prevents late rises in postprandial TGs and TNF- α , thus improving cardiovascular risk profile.

45 **Clinical trial registration:** clinicaltrials.gov; Reg. no. NCT02595658

46 **Keywords:**

47 Type 1 diabetes, high-fat feeding, lipaemia, inflammation, cardiovascular risk

48

49

50 **Introduction**

51 Structured education provided to patients with type 1 diabetes for managing meal-time insulin
52 dose focuses on the carbohydrate-counting method ^{1,2}, whereby people calculate the dose of
53 insulin administered at meal-times based on the total carbohydrate content of that meal ³.
54 Whilst this has been demonstrated as an effective strategy for HbA_{1c} reduction ³, typical eating
55 patterns consist of the consumption of *mixed-macronutrient* meals ⁴, and in reality many people
56 with type 1 diabetes still struggle to maintain postprandial euglycaemia ⁵. This is particularly
57 the case for individuals treated with modern insulin analogue injections, as this method of
58 insulin delivery is associated with less meal-time insulin dose flexibility compared to
59 Continuous Subcutaneous Insulin Infusion therapy (CSII) ⁶.

60

61 The addition of fat to a carbohydrate-based meal has been shown to cause postprandial
62 hyperglycaemia, and increase insulin requirements late into the postprandial period ^{7, 8}. In
63 clinical practice, people with type 1 diabetes are often reluctant to administer an additional
64 injection of bolus insulin either at mealtime or late into the postprandial period for fear of
65 hypoglycaemia or because increasing injection frequency is considered to be burdensome ^{6,9}.
66 Recently, we have showed that when consuming a carbohydrate-based meal with a high-fat
67 content, adopting the carbohydrate-counting method for insulin dose adjustments at meal-time
68 followed by the administration of an additional insulin-bolus late into the postprandial period
69 is important for the normalisation of glycaemia ⁷. Specifically, compared to the carbohydrate-
70 counting method alone, administration of additional bolus insulin 3 hours later resulted in a
71 23% reduction in blood glucose area under the curve (AUC) ⁷. Importantly, this method did
72 not cause hypoglycaemia, whereas simply increasing the amount of rapid-acting insulin dose
73 administered at meal-time did ⁷.

74

75 Insulin has an important role not only in the control of postprandial glucose excursions, but
76 also in the regulation of postprandial lipaemia¹⁰. Excessive increases in both glycaemia and
77 lipaemia can create a pro-inflammatory and -coagulant milieu¹¹⁻¹⁵, and are collectively and
78 independently associated with cardiovascular disease (CVD) and early mortality^{16, 17}.
79 Considering the substantial pre-existing risk of CVD-associated early mortality in this cohort
80^{18, 19}, and the potential for this to be heightened by exaggerated post-prandial lipaemia²⁰⁻²⁴,
81 optimising meal time insulin dosage is important for cardiovascular risk management, not just
82 normalisation of glycaemia *per se*. However, the influence of administering additional insulin
83 late into the postprandial period on metabolic or cardiovascular risk factors in patients with
84 type 1 diabetes treated with basal-bolus injection therapy has never been assessed. In this study,
85 we manipulated rapid-acting insulin injection dosage and timing in response to a high-
86 carbohydrate high-fat meal feeding to test the hypothesis that an additional but delayed rapid-
87 acting insulin bolus is required to normalise postprandial lipaemia and the associated
88 metabolic, inflammatory, and pro-coagulant response.

89

90 **Methods**

91 **Patients**

92 The study population consisted of 10 male type 1 diabetes patients (mean \pm SD; age 26 ± 4
93 years, BMI 25.4 ± 1.6 kg.m², duration of diabetes 17 ± 5 years, age at diagnosis 9 ± 4 years;
94 HbA_{1c} 52.5 ± 5.9 mmol/mol [7.0 ± 0.5 %]). Patients were eligible for inclusion if they were
95 aged between 18-50 years, with a duration of diabetes greater than 5 years on enrolment, treated
96 on basal-bolus insulin regimen, and were familiar with carbohydrate counting and using a
97 stable insulin-to-carbohydrate ratio. Patients were treated on a stable basal-bolus insulin
98 analogue regimen consisting of either insulin glargine ($n = 8$) or detemir ($n = 2$) and fast-acting
99 insulin analogue aspart ($n = 10$), for a minimum of 12 months. Patients were free of diabetes

100 related complications, and were receiving no additional medication other than insulin. All
101 patients had received structured education in carbohydrate counting as part of their diabetes
102 care. This study received approval by the local National Health Service Research Ethics
103 Committee (R&D Ref: 7241). All patients who participated provided written informed consent.
104 Eligible patients underwent randomization by computer program to determine the sequence of
105 3 crossover conditions.

106

107 **Pre-Laboratory Phase**

108 Patients arrived at the laboratory after an overnight fast (>10 hours) having replicated their diet
109 in the previous 48 hours (assessed using weighed dietary recording sheets). Participants were
110 fitted with a real-time continuous glucose monitor (CGM; Paradigm Veo, Medtronic Diabetes,
111 Northridge, CA) as described previously ^{7, 11, 25-27} to aid in the maintenance of normal
112 glycaemia during the pre-laboratory period. Additionally, patients were instructed to maintain
113 their normal insulin regimen, with basal insulin dose standardized (dose, injection site, time of
114 injection) across conditions. Patients were also given a pedometer (Omron Healthcare Europe
115 B.V., Netherlands), which they were instructed to wear over the course of 24 hours prior to
116 experimental visits. Patients were required to avoid strenuous activity in the previous 48 hours
117 and maintain similar activity patterns between visits.

118

119 **Main Experimental Visits**

120 In a randomised and counter-balanced fashion participants attended three separate morning
121 time (~07:00AM) laboratory-based visits, each interspersed by seven days. Upon arrival to the
122 laboratory, patients assumed a seated and rested position whilst a 20-gauge cannula (Vasofix,
123 B. Braun, Melsungen AG, Melsungen, Germany) was inserted into the antecubital vein of their

124 non-dominant arm; resting, fasted, venous blood samples were then collected prior to
125 experimental testing.

126

127 Each experimental visit involved the consumption of meals matched for carbohydrate and
128 protein content, but differing in 1) fat content and 2) rapid-acting insulin bolus dose and timing.

129 The **LF** condition involved administration of rapid-acting insulin according to individual
130 patient carbohydrate counting requirements (dose per 10 g: $1.1 \pm 0.8\text{IU}$) prior to the
131 consumption of a low-fat meal (Table 1). The **HF** condition involved administration of rapid-
132 acting insulin according to the individual patient carbohydrate counting requirements (as
133 administered in **LF**), however, the meal contained an additional 50 g of fat to constitute a high-
134 fat meal (Table 1). The **HFA** condition involved the administration of rapid-acting insulin
135 according to individual carbohydrate counting requirements (as administered in **LF** and **HF**)
136 prior to the consumption of a high-fat meal, and an additional 30% of rapid-acting insulin
137 administered at 180 minutes post-ingestion (Table 1). The aim of this was such that the
138 additional units of insulin would enter the circulation to coincide with the occurrence of peak
139 postprandial lipaemia²⁸.

140

141 To minimise the influence of injection location on insulin absorption kinetics, the site of bolus
142 injection was standardised across visits using prominent anatomical landmarks (equidistant
143 from the most medial portion of the iliac crest and navel).

144

145 Following meal consumption patients remained in a seated and rested position with blood
146 samples drawn every 30 minutes for 6 hours following meal consumption. Following this,
147 patients were discharged and returned home. Plasma Fibrinogen, Human Tissue Factor (HTF),

148 and Plasminogen Activator Inhibitor-1 (PAI-1) were sampled at baseline, 3 hours post-meal,
149 and at 6 hours post-meal.

150

151 **Meal Composition**

152 The macronutrient contribution to each meal is presented in Table 1; meal carbohydrate and
153 protein content were matched across conditions. The low-fat and high-fat meals were based
154 upon the composition of a curried dish consisting of basmati rice (Basmati Rice Basics, Tesco,
155 UK), tikka masala sauce (Mild Spice Tikka Masala, Weight Watchers, UK), and chicken
156 (Everyday Sliced Chicken, Tesco, UK). The amount of each food item was identical in each
157 condition and calculated such that carbohydrate content was individualised, equalling 1 g of
158 carbohydrate per Kg of body mass. In addition to the above, the high-fat meals included the
159 addition of an absolute amount of 50 g of clarified butter (Butter Ghee, East End Foods, UK)
160 which constitutes 99.9% fat.

161

162 **Blood Sampling**

163 At each time point 10 ml of venous whole blood was taken and dispensed into serum separation
164 and lithium-heparin (Vacuette, Greiner Bio-One GmbH, Kremsmünster, Austria) tubes before
165 being centrifuged for 15 minutes at 2,000 g at 4°C and stored at -80°C for retrospective analysis
166 of Triglycerides (TG; Serum Triglyceride Determination Kit; Sigma-Aldrich, St. Louis, MO,
167 USA). Apolipoprotein B48 (APO_{B48}; Apolipoprotein B48, Antibodies-online, USA), Non-
168 Esterified-Fatty Acids (NEFA; RANBUT, Randox Laboratories, London, UK), plasma
169 Glucagon (Glucagon EIA, Sigma-Aldrich, St. Louis, MO, USA), Tumour Necrosis Factor
170 alpha (TNF- α ; Human TNF- α Quantikine ELISA, R&D Systems, Roche Diagnostics, West
171 Sussex, UK) were measured hourly. Plasma Fibrinogen (ab108842, Fibrinogen Human ELISA
172 Kit, Abcam, Japan), HTF (Human Tissue Factor activity ab108906, abcam, UK), and PAI-1

173 (Human PAI-1/serpin ELISA Kit DSE100, R7D systems, UK) were measured at Rest, 3 hours
174 and 6 hours post-meal. The intra-assay coefficient of variation was < 10% for all assays. Due
175 to increased assay cross-reactivity with insulin detemir, only participants treated with insulin
176 glargine were included in serum insulin analysis ($n = 8$).

177

178 **Data Analysis**

179 Sample size analyses was performed using data from Cohen and Berger²⁹, whereby increasing
180 insulinaemia via the co-ingestion of glucose lowered postprandial (3 hours) TG concentrations
181 from $\sim 1.36 \pm 0.24 \text{ mmol.l}^{-1}$ to $0.85 \pm 0.24 \text{ mmol.l}^{-1}$. Based on these data, 10 participants should
182 provide >90% chance of statistically detecting a similar effect size with an α -level of 0.05.
183 Statistical analysis was performed using PASW Statistics 18 software (IBM, Armonk, NY)
184 with significance set at $p \leq 0.05$. Data were examined using repeated measures ANOVA
185 (condition*time). Where significant p -values were identified for interaction effects
186 (condition*time), Bonferroni corrected post-hoc analysis was performed. Significant main
187 effects of time were investigated using pairwise comparisons. Where relevant, one-way
188 ANOVA with Bonferroni adjusted pairwise comparisons was used to compare between
189 conditional differences. Data are presented as mean \pm SD unless stated otherwise.

190

191 ***** INSERT TABLE 1 *****

192

193 **Results**

194 **Pre-Laboratory Phase**

195 Patients displayed similar glycaemic control during the 24 hours before arriving to the
196 laboratory, with similar mean (**LF** 7.5 ± 1.6 , **HF** 7.0 ± 1.1 , **HFA** $8.2 \pm 1.5 \text{ mmol.l}^{-1}$; $p = 0.519$) and
197 total AUC (**LF** 11123 ± 2224 , **HF** $10,080 \pm 1543$, **HFA** $11762 \pm 2159 \text{ mmol.l}^{-1} \cdot \text{min}^{-1}$; $p = 0.328$)

198 interstitial glucose across visits. Throughout this time, patients demonstrated similar dietary
199 patterns, rapid-acting insulin administrations, and activity levels across conditions ($p > 0.05$;
200 Supplement 1).

201

202 **Laboratory Phase**

203 TG concentrations following **LF** remained similar to baseline (Figure 1A; $p > 0.05$), whereas
204 TGs under **HF** were significantly greater throughout the 360 minute observation period (Figure
205 1A, B, C; $p < 0.05$). **HFA** elicited an increase in TGs similar to **HF** concentrations during the
206 first 180 minutes, but beyond 180 minutes concentrations returned to baseline and were
207 comparable to **LF** (Figure 1A, B, C; $p > 0.05$). **HF** was also associated with elevated TNF- α
208 late into the postprandial period, whereas **LF** and **HFA** was not (Figure 2A, B; $p < 0.05$). The
209 CGM interstitial glucose responses are presented in Figure 2C. There was a significant
210 time*condition interaction ($p = 0.02$, partial- $\eta^2 = 0.199$), and a significant time ($p < 0.01$,
211 partial- $\eta^2 = 0.753$) and condition ($p = 0.29$, partial- $\eta^2 = 0.324$) effect in CGM interstitial glucose
212 responses to the conditions (Figure 2C), whereby **HF** resulted in higher interstitial glucose
213 concentrations in the late postprandial period compared to both **LF** and **HFA** (Figure 2C; $p <$
214 0.05). Interstitial glucose was comparable between conditions during the first 180 minutes with
215 similar total AUC₀₋₁₈₀ (**LF** 4104 \pm 831, **HF** 5401 \pm 545, **HFA** 4959 \pm 525 mmol.l⁻¹.min⁻¹; $p =$
216 0.418) and absolute interstitial glucose levels at 180 minutes (**LF** 6.0 \pm 1.3, **HF** 7.0 \pm 0.7, **HFA**
217 6.3 \pm 0.7 mmol.l⁻¹; $p > 0.05$; Figure 2C). Beyond 180 minutes, interstitial glucose levels were
218 greater under **HFA** (AUC₁₈₀₋₃₆₀: **LF** 8518 \pm 1876, **HF** 14,591 \pm 1957 vs. **HFA** 11,011 \pm 1509
219 mmol.l⁻¹.min⁻¹; $p < 0.05$). The APO_{B48}, NEFA, Glucagon, fibrinogen, HTF activity, and PAI-
220 1 responses are presented in Table 2.

221

222

223 *** INSER FIGURE 1 ***

224 *** INSER FIGURE 2 ***

225 *** INSER TABLE 1 ***

226

227 Discussion

228 This is the first study to show that in adult type 1 diabetes patients treated with modern insulin
229 analogue injections, an additional rapid-acting insulin dose, provided 3 hours after ingestion of
230 a high-carbohydrate high-fat meal, reduces the late rise in lipaemia seen with when the
231 carbohydrate counting method for insulin administration at meal time is used alone. Moreover,
232 such a strategy provides a similar postprandial glycaemic and inflammatory response to a meal
233 with negligible fat content and does not augment the pro-coagulant response of fibrinogen,
234 HFP or PAI-1. In comparison, when following the carbohydrate counting method at meal time
235 alone patients are likely to experience raised lipaemia, hyperglycaemia, and elevated TNF- α
236 concentrations late into the post-prandial period. These findings further highlight the
237 importance of an additional but delayed insulin bolus not just for glucose control *per se*, but
238 for normalisation of a milieu potentially promoting vascular damage.

239

240 Our data show that the addition of dietary fat increases rapid-acting insulin dose requirements,
241 similar to that shown previously in patients using (CSII)^{8, 30-33}. For example, Wolpert et al.⁸
242 showed that under closed-loop glucose control, the insulin requirement for a high-fat evening
243 meal was increased by ~42%, in comparison to a carbohydrate-matched, low-fat meal. The
244 present study furthers these previous findings by examining how adjusting the dose and timing
245 of rapid-acting insulin administration influences the metabolic milieu and cardiovascular risk
246 factors associated with consuming mixed macronutrient meals; to date has not been examined
247 within the literature. Our data demonstrate that when administering rapid-acting insulin to

248 cover only the carbohydrate content of the meal (as in the **HF** condition) patients are exposed
249 to raised triglycerides and TNF- α at 4-6 hours post-meal (Figure 1A-C, Figure 1A). In addition,
250 we observed a trend towards an increase in fibrinogen late into the postprandial period; our
251 sample size was likely too small for yield statistical significance in this individual marker,
252 however our findings indicate an increased inflammatory *and* thrombotic response following
253 high-carbohydrate, high-fat meal feeding in people with type 1 diabetes that can be prevented
254 with an additional delayed bolus of insulin. These data call for a larger scale observation of the
255 thrombotic responses to high-carbohydrate high-fat meal feeding, and it is recommended that
256 subsequent interventions to reduce post-prandial lipaemia consider this as a potentially
257 important outcome.

258

259 Prior research has shown that high-fat meals (> 70 grams of fat) can increase pro-coagulation
260 markers ^{34, 35}, however in the measures we chose, we saw no influence of meal type or dosing
261 strategy. The fat content of the meals within this study was chosen such that they replicated
262 meals that may habitually be consumed by patients (~50 grams of fat), and may simply not
263 have been large enough for subtle changes in insulin dose (+30% equalling ~2.6 IU) and timing
264 to cause a demonstrable effect at the respective sample points. Additionally, the postprandial
265 glucose excursions were only moderately hyperglycaemic under both high-fat conditions.

266

267 It is noteworthy that foods with different fatty acid profiles may elicit different postprandial
268 lipaemic ²⁸ and inflammatory ³⁶ responses, potentially mediated via modulation of insulin
269 sensitivity ³⁷, gastric emptying ³⁸, gut hormones responses ³⁸, circulating adhesion molecules
270 ³⁹, and oxidative stress generation ^{39, 40}. Fats predominantly saturated and of long-chain in
271 composition cause a delayed postprandial lipaemic response ^{28, 34}. The fatty acid profile of the
272 clarified butter added to the meals in the present study was ~62% saturated and 29%

273 monounsaturated fat, which, as highlighted in this study is likely to result in a delayed and
274 exaggerated lipaemic response occurring beyond the action time profiles of modern rapid-
275 acting insulin analogues if administered as a single bolus at the time of meal ingestion ⁴¹. As
276 such, the differential responses between **HF** and **HFA** in late lipaemia can be attributed to our
277 insulin administration strategy, considering i) glycaemia was similar between conditions up to
278 180 minutes post-meal, and ii) the triglyceride response under **HF** beyond 180 minutes is
279 comparable to previous observations profiling time-course lipaemic responses in individuals
280 without type 1 diabetes following high-fat feeding ⁴².

281

282 Prior research examining the interactions of protein in isolation ^{43,44} and in combination with
283 carbohydrate and fat ³², shows that protein can raise postprandial glucose late after feeding,
284 with additive effects when combined with fat ³². Meal protein content was kept under 30 g,
285 such that no bolus insulin dose adjustment for the protein content would be needed ⁴⁵; indeed,
286 under the **LF** condition, patients demonstrated no late postprandial hyperglycaemia, with all
287 patients remaining within euglycaemic ranges when the carbohydrate counting method was
288 employed.

289

290 **Conclusions**

291 In conclusion, these are the first data to demonstrate that when eating a meal with a high-
292 carbohydrate and high-fat content, an additional insulin dose provided 3 hours into the
293 postprandial period reduces plasma triglyceride concentrations and inflammatory markers in
294 type 1 diabetes patients. Thus people with type 1 diabetes treated with basal-bolus insulin
295 injections should be encouraged to carbohydrate count at meal time and administer additional
296 insulin units 3 hours into the postprandial period when consuming a high-carbohydrate, high-
297 fat meal. Not accounting for the fat component of the meal is associated with raised blood

298 lipids, delayed glucose excursions, and increased inflammation. Based on our findings, patients
299 should be advised of the importance of the late bolus not just for glucose control, but for also
300 normalising other markers that may negatively influence vascular health.

301

302 **List of abbreviations**

303 APO_{B48} = Apolipoprotein B48; AUC = Area Under the Curve; BMI = Body Mass Index; CGM
304 = Continuous Glucose Monitoring; CSII Continuous Subcutaneous Insulin Infusion; CVD =
305 Cardiovascular Disease; HF = High-Fat; HFP = Human Tissue Factor; HFA = High-Fat Split;
306 LF= Low-Fat; NEFA = Non-Esterified Fatty Acids; TG = Triglycerides; TNF- α = Tumor
307 Necrosis Factor Alpha

308 **Figure legends**

309 **Figure 1 A-C.** **A** Time course changes in plasma triglycerides; **B** Total plasma triglyceride
310 AUC₀₋₁₈₀; **C** Total plasma triglyceride AUC₁₈₀₋₃₆₀. Red trace/bar = **HF**; Blue trace / bar = **HFA**;
311 Black trace/bar = **LF**. Data presented as mean \pm SD. * indicates significantly different to **LF**, **
312 indicates significantly different to **LF** and **HFA**. Dashed line break on panel B indicates
313 additional insulin bolus administration.

314

315 **Figure 2 A-C.** **A** Time course changes in TNF- α ; **B** Total plasma TNF- α AUC₁₈₀₋₃₆₀; **C** Time
316 course changes in CGM interstitial glucose. Red bar/trace = **HF**; Blue bar/trace = **HFA**; Black
317 bar/trace = **LF**. Data presented as mean \pm SD. CGM data presented as mean \pm SEM for reader
318 clarity. * indicates a significantly different to **LF**, ** indicates significantly different to **LF** and
319 **HFA**. Dashed line break on panel B indicates additional insulin bolus administration.

Tables

Table 1. Experimental meal composition and accompanying insulin administration

		LF	HF	HFA
Energy	MJ	4±0	4±0	4±0
Carbohydrate	%E	34	34	34
	g	68±3	68±3	68±3
Fat	%E	10	55	55
	g	5±0	58±2	58±2
Protein	%E	11	11	11
	g	26±1	26±1	26±1
Total Insulin Administration (IU)	IU	9±2	9±2	9±2 + 3±1

Note: Data are presented as mean ± SD; $n = 10$. All meals composed of 1 g carbohydrate Kg body mass. All meals were composed equally of basmati rice (Tesco, UK), chicken breast (Tesco, UK), and a low fat curry sauce (Tikka Masala Sauce, Weight watchers, UK). **HF** and **HFA** contained an additional 50 g of fat in the form of clarified butter (Ghee, East End Foods, UK). **%E** = percentage of energy intake.

Table 2. Responses of metabolic, hormonal, inflammatory, chylomicron, and coagulation markers following high-fat meals / insulin administration

		ANOVA <i>p</i>							T	T*C
		Rest	60	120	180	240	300	360		
APO_{B48} (mg.ml ⁻¹)	LF	6.65±5.98	7.72±5.52	8.16±4.98	8.75±5.68	10.90±9.61	10.98±8.43	11.27±13.53	=0.410	=0.267
	HF	4.93±2.94	7.25±6.83	6.88±7.27	9.56±8.82	14.52±14.92	9.69±13.91	12.28±10.52		
	HFA	6.06±5.96	9.85±7.14	7.50±5.36	9.91±10.34	10.93±10.16	9.59±10.01	11.59±17.21		
NEFA (mmol.l ⁻¹)	LF	0.39±0.21	0.21±0.06†	0.14±0.10†*	0.17±0.11†*	0.24±0.14†‡*	0.36±0.13†‡*	0.41±0.15	<0.001	<0.001
	HF	0.47±0.33	0.20±0.10†	0.26±0.07†	0.36±0.10†	0.41±0.18†*	0.41±0.19†*	0.38±0.18†		
	HFA	0.52±0.20	0.22±0.10†	0.30±0.11†	0.40±0.14†	0.43±0.21†	0.28±0.13†‡*	0.36±0.13†		
Glucagon (pg.ml ⁻¹)	LF	482±128	502±150	493±148	498±102	475±97	432±47	465±64	=0.195	=0.700
	HF	471±160	500±167	524±164	498±164	458±156	449±156	440±156		
	HFA	467±135	480±150	498±152	483±127	453±121	428±85	438±102		
Fibrinogen (ug.ml ⁻¹)	LF	2326±1131	---	---	2360±2184	---	---	2300±2268	=0.056	=0.398
	HF	1988±1385	---	---	3314±3191	---	---	4436±5388		
	HFA	2286±1094	---	---	3660±5750	---	---	3346±3075		
HTF Activity (pmol.ml ⁻¹)	LF	131.74±61.53	---	---	183.71±81.73	---	---	119.02±44.79	=0.087	=0.328
	HF	124.18±68.89	---	---	192.69±76.55	---	---	129.42±35.94		
	HFA	134.00±62.65	---	---	191.02±110.96	---	---	218.30±64.84		
PAI-1 (ng.ml ⁻¹)	LF	1.34±0.90	---	---	1.41±0.72	---	---	1.33±0.62	=0.311	=0.100
	HF	0.92±0.60	---	---	1.01±0.40	---	---	1.88±1.46		
	HFA	1.00±0.62	---	---	1.25±1.15	---	---	2.63±4.67		

Note: Data presented as mean±SD (*n* = 10). * indicates significantly different from **HF**, ** indicates significantly different from **HF** and **LF**, † indicates significantly different from rest, ‡ indicates significantly different from 180 minutes. T = time effect, T*C = time X condition interaction effect.

Declarations

Ethics approval and consent to participate

This study received approval by the local National Health Service Research Ethics Committee (R&D Ref: 7241). All patients who participated provided written informed consent.

Consent for publication

Not applicable – no presentation of individual data

Availability of data and material

All data generated or analysed during this study are included in the published article [and its supplementary information films]

Competing interests

The authors declare that they have competing interests

Funding

This study was funded by Newcastle University. Only the named research team were involved in the design of the study, collection, analysis, and interpretation of data, and in writing the manuscript

Authors' contributions

MDC designed the study, collected, analysed and interpreted data, and wrote the manuscript. MW assisted in data collection and prepared the manuscript. RAA contributed to the interpretation of data and preparation of the manuscript. KMB contributed to the interpretation of data and preparation of the manuscript. JTG designed the study, collected, and interpreted

data, and wrote the manuscript. DJW designed the study, analysed and interpreted data, and wrote the manuscript.

Acknowledgements

The authors wish to acknowledge the time and commitment of the study participants, and the Research Team at the Newcastle NIHR Clinical Research Facility in assisting in trial management and study conduct.

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