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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 ( $HbA_{1c}$   $52.5 \pm 5.9$  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, APO<sub>B48</sub>, glucagon, TNF-  
36  $\alpha$ , 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- $\alpha$ , 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- $\alpha$ , thus improving cardiovascular risk profile.

45 **Clinical trial registration:** [clinicaltrials.gov](https://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 <sup>1,2</sup>, whereby people calculate the dose of  
53 insulin administered at meal-times based on the total carbohydrate content of that meal <sup>3</sup>.  
54 Whilst this has been demonstrated as an effective strategy for HbA<sub>1c</sub> reduction <sup>3</sup>, typical eating  
55 patterns consist of the consumption of *mixed-macronutrient* meals <sup>4</sup>, and in reality many people  
56 with type 1 diabetes still struggle to maintain postprandial euglycaemia <sup>5</sup>. 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) <sup>6</sup>.

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 <sup>7, 8</sup>. 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 <sup>6,9</sup>.  
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 <sup>7</sup>. 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) <sup>7</sup>. Importantly, this method did  
72 not cause hypoglycaemia, whereas simply increasing the amount of rapid-acting insulin dose  
73 administered at meal-time did <sup>7</sup>.

74

75 Insulin has an important role not only in the control of postprandial glucose excursions, but  
76 also in the regulation of postprandial lipaemia<sup>10</sup>. Excessive increases in both glycaemia and  
77 lipaemia can create a pro-inflammatory and -coagulant milieu<sup>11-15</sup>, and are collectively and  
78 independently associated with cardiovascular disease (CVD) and early mortality<sup>16, 17</sup>.  
79 Considering the substantial pre-existing risk of CVD-associated early mortality in this cohort  
80<sup>18, 19</sup>, and the potential for this to be heightened by exaggerated post-prandial lipaemia<sup>20-24</sup>,  
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  $26 \pm 4$   
93 years, BMI  $25.4 \pm 1.6$  kg.m<sup>2</sup>, duration of diabetes  $17 \pm 5$  years, age at diagnosis  $9 \pm 4$  years;  
94 HbA<sub>1c</sub>  $52.5 \pm 5.9$  mmol/mol [ $7.0 \pm 0.5$  %]). 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 <sup>7, 11, 25-27</sup> 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 lipaemia<sup>28</sup>.

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 (Vacurette, 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<sub>B48</sub>; 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- $\alpha$ ; Human TNF- $\alpha$  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 Berger<sup>29</sup>, 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  $\alpha$ -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**  $7.5 \pm 1.6$ , **HF**  $7.0 \pm 1.1$ , **HFA**  $8.2 \pm 1.5 \text{ mmol.l}^{-1}$ ;  $p = 0.519$ ) and  
197 total AUC (**LF**  $11123 \pm 2224$ , **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- $\alpha$   
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 AUC<sub>0-180</sub> (**LF** 4104 $\pm$ 831, **HF** 5401 $\pm$ 545, **HFA** 4959 $\pm$ 525 mmol.l<sup>-1</sup>.min<sup>-1</sup>;  $p =$   
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<sup>-1</sup>;  $p > 0.05$ ; Figure 2C). Beyond 180 minutes, interstitial glucose levels were  
218 greater under **HFA** (AUC<sub>180-360</sub>: **LF** 8518 $\pm$ 1876, **HF** 14,591 $\pm$ 1957 vs. **HFA** 11,011 $\pm$ 1509  
219 mmol.l<sup>-1</sup>.min<sup>-1</sup>;  $p < 0.05$ ). The APO<sub>B48</sub>, NEFA, Glucagon, fibrinogen, HTF activity, and PAI-  
220 1 responses are presented in Table 2.

221

222

223 \*\*\* INSERT FIGURE 1 \*\*\*

224 \*\*\* INSERT FIGURE 2 \*\*\*

225 \*\*\* INSERT 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- $\alpha$   
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) <sup>8, 30-33</sup>. For example, Wolpert et al. <sup>8</sup>  
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- $\alpha$  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 <sup>34, 35</sup>, 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 <sup>28</sup> and inflammatory <sup>36</sup> responses, potentially mediated via modulation of insulin  
269 sensitivity <sup>37</sup>, gastric emptying <sup>38</sup>, gut hormones responses <sup>38</sup>, circulating adhesion molecules  
270 <sup>39</sup>, and oxidative stress generation <sup>39, 40</sup>. Fats predominantly saturated and of long-chain in  
271 composition cause a delayed postprandial lipaemic response <sup>28, 34</sup>. 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 <sup>41</sup>. 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 <sup>42</sup>.

281  
282 Prior research examining the interactions of protein in isolation <sup>43,44</sup> and in combination with  
283 carbohydrate and fat <sup>32</sup>, shows that protein can raise postprandial glucose late after feeding,  
284 with additive effects when combined with fat <sup>32</sup>. Meal protein content was kept under 30 g,  
285 such that no bolus insulin dose adjustment for the protein content would be needed <sup>45</sup>; 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<sub>B48</sub> = 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- $\alpha$  = 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 AUC<sub>0-180</sub>; **C** Total plasma triglyceride AUC<sub>180-360</sub>. 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- $\alpha$ ; **B** Total plasma TNF- $\alpha$  AUC<sub>180-360</sub>; **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

		<b>LF</b>	<b>HF</b>	<b>HFA</b>
<b>Energy</b>	<b>MJ</b>	4±0	4±0	4±0
<b>Carbohydrate</b>	<b>%E</b>	34	34	34
	<b>g</b>	68±3	68±3	68±3
<b>Fat</b>	<b>%E</b>	10	55	55
	<b>g</b>	5±0	58±2	58±2
<b>Protein</b>	<b>%E</b>	11	11	11
	<b>g</b>	26±1	26±1	26±1
<b>Total Insulin Administration (IU)</b>	<b>IU</b>	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		
<b>APO<sub>B48</sub></b> (mg.ml <sup>-1</sup> )	<b>LF</b>	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
	<b>HF</b>	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		
	<b>HFA</b>	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		
<b>NEFA</b> (mmol.l <sup>-1</sup> )	<b>LF</b>	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
	<b>HF</b>	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†		
	<b>HFA</b>	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†		
<b>Glucagon</b> (pg.ml <sup>-1</sup> )	<b>LF</b>	482±128	502±150	493±148	498±102	475±97	432±47	465±64	=0.195	=0.700
	<b>HF</b>	471±160	500±167	524±164	498±164	458±156	449±156	440±156		
	<b>HFA</b>	467±135	480±150	498±152	483±127	453±121	428±85	438±102		
<b>Fibrinogen</b> (ug.ml <sup>-1</sup> )	<b>LF</b>	2326±1131	---	---	2360±2184	---	---	2300±2268	=0.056	=0.398
	<b>HF</b>	1988±1385	---	---	3314±3191	---	---	4436±5388		
	<b>HFA</b>	2286±1094	---	---	3660±5750	---	---	3346±3075		
<b>HTF Activity</b> (pmol.ml <sup>-1</sup> )	<b>LF</b>	131.74±61.53	---	---	183.71±81.73	---	---	119.02±44.79	=0.087	=0.328
	<b>HF</b>	124.18±68.89	---	---	192.69±76.55	---	---	129.42±35.94		
	<b>HFA</b>	134.00±62.65	---	---	191.02±110.96	---	---	218.30±64.84		
<b>PAI-1</b> (ng.ml <sup>-1</sup> )	<b>LF</b>	1.34±0.90	---	---	1.41±0.72	---	---	1.33±0.62	=0.311	=0.100
	<b>HF</b>	0.92±0.60	---	---	1.01±0.40	---	---	1.88±1.46		
	<b>HFA</b>	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

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### **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.

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