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Glucose-dependent Insulinotropic Polypeptide promotes lipid deposition in subcutaneous adipocytes in obese, type 2 diabetes patients: a maladaptive response

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38 **Abstract**

39 Glucose-dependent insulintropic polypeptide (GIP) beyond its insulintropic effects may
40 regulate post-prandial lipid metabolism. While the insulintropic action of GIP is known to
41 be impaired in type 2 diabetes mellitus (T2DM), its adipogenic effect is unknown. We
42 hypothesised GIP is anabolic in human subcutaneous adipose tissue (SAT) promoting
43 triacylglycerol (TAG) deposition through re-esterification of non-esterified fatty acids
44 (NEFA) and this effect may differ according to obesity status or glucose tolerance.

45 **Methods:** 23 subjects, categorised in four groups: normoglycaemic lean (n=6),
46 normoglycaemic obese, (n=6), obese with impaired glucose regulation (IGR) (n=6) and
47 obese, T2DM (n=5) participated in a double-blind, randomised, crossover study involving a
48 hyperglycaemic clamp with a 240 minute GIP infusion ($2\text{pmol kg}^{-1}\text{min}^{-1}$) or normal saline.
49 Insulin, NEFA, SAT-TAG content and gene expression of key lipogenic enzymes were
50 determined before and immediately after GIP/saline infusions.

51 **Results:** GIP lowered NEFA concentrations in obese T2DM group despite diminished
52 insulintropic activity (mean NEFA $\text{AUC}_{0-4\text{hr}} \pm \text{SEM}$, $41992 \pm 9843 \mu\text{mol/L/min}$ vs 71468
53 ± 13605 with placebo, $p=0.039$; 95% CI 0.31 to 0.95). Additionally, GIP increased SAT-TAG
54 in obese T2DM (1.78 ± 0.4 vs 0.86 ± 0.1 fold with placebo, $p=0.043$; 95% CI: 0.1 to 1.8).
55 Such effect with GIP was not observed in other three groups despite greater insulintropic
56 activity. Reduction in NEFA concentration with GIP correlated with adipose tissue insulin
57 resistance for all subjects (Pearson $r=0.56$, $p=0.005$). There were no significant gene
58 expression changes in key SAT lipid metabolism enzymes.

59 **Conclusion:** GIP appears to promote fat accretion and thus may exacerbate obesity and
60 insulin resistance in T2DM.

61 **Key words:** GIP, type 2 diabetes, adipose tissue, lipid metabolism, NEFA

62

63 **Introduction**

64 In healthy individuals, glucose-dependent insulintropic polypeptide (GIP) is secreted from
65 small intestinal K cells in response to intraluminal carbohydrate, protein and most potently
66 fat; GIP in turn stimulates (glucose-dependent) pancreatic insulin secretion. However, in
67 patients with type 2 diabetes mellitus (T2DM), despite preserved GIP secretion (11) the
68 insulintropic action of GIP is severely impaired (12, 16, 35).

69

70 GIP has other important extra-pancreatic metabolic functions with receptors expressed in
71 such tissues as bone, brain, stomach and adipose tissue, where it may modulate post-prandial
72 lipid metabolism (7). In animal models of obesity-induced insulin resistance, genetic and
73 chemical disruption of GIP signaling protects against the deleterious effects of high fat
74 feeding by preventing lipid deposition, adipocyte hypertrophy and expansion of adipose
75 tissue mass, and reducing triglyceride deposition in liver and skeletal muscle, maintaining
76 insulin sensitivity (25, 31). Thus if GIP has a potential pro-adipogenic effect, selective GIP
77 antagonists may be beneficial in treating obesity and type 2 diabetes mellitus (T2DM) (17).

78

79 There is evidence that plasma GIP concentrations are increased in obesity. Given that dietary
80 fat consumption chronically stimulates the production and secretion of GIP, inducing K cell
81 hyperplasia (8, 36), higher GIP concentrations may reflect consumption of an energy dense,
82 high-fat diet. Early rodent studies demonstrated that a GIP infusion, during an intraduodenal
83 lipid infusion, decreased plasma triglyceride levels (14) while GIP has been shown to
84 enhance insulin-induced fatty acid incorporation in rat adipose tissue (9). Thus GIP, mediated
85 through the adipocyte GIP receptor, is anabolic in adipose tissue promoting fat deposition.

86

87 It is important to distinguish between direct effects of GIP on fatty acid metabolism and
88 indirect effects based on its insulinotropic action. Acute GIP infusion in lean healthy males
89 (with hyperinsulinaemia and hyperglycaemia) increases adipose tissue blood flow,
90 triacylglycerol (TAG) hydrolysis and FFA re-esterification thus promoting triglyceride
91 deposition (5, 6). In healthy obese men, acute GIP infusion reduced expression and activity of
92 11β hydroxysteroid dehydrogenase type 1 (11β -HSD1), a fat-specific glucocorticoid
93 metabolism enzyme that may enhance lipolysis in subcutaneous adipose tissue (SAT) (20). In
94 addition, it has been suggested that GIP contributes to induction of adipocyte and SAT
95 inflammation (and thus insulin resistance), increasing production of pro-inflammatory
96 adipokines such as monocyte chemoattractant protein-1 (MCP-1) (21), IL-6, IL- 1β and
97 osteopontin (1, 37). Thus from the available animal model and human data, GIP appears to
98 have a key regulatory role in lipid metabolism and adipose tissue.

99

100 To date, very few studies have investigated the effects of GIP on human adipose tissue and
101 none have involved subjects with T2DM although the reported presence of functional GIP
102 receptors on adipocytes strongly suggests GIP modulates human adipose tissue metabolism
103 (41). GIP has also been proposed to modulate other adipose tissue depots, and that excessive
104 GIP secretion may underlie excessive visceral and liver fat deposition (33, 34). In support of
105 this, results from a cross-sectional study of Danish men demonstrated an association between
106 higher levels of GIP (during a glucose tolerance test) and a metabolically unfavourable
107 phenotype (higher visceral: subcutaneous fat and a higher waist-hip ratio) (32).

108

109 We hypothesized that GIP would have an anabolic action in SAT promoting FFA re-
110 esterification, which we speculated may be mediated either by enhancing lipoprotein lipase
111 (LPL) expression/activity (a lipogenic enzyme), (15, 26) or by reducing adipose tissue

112 triglyceride lipase (ATGL) and hormone sensitive lipase (HSL) expression/activity, two key
113 lipolytic enzymes. We postulated that this effect may be different according to obesity status
114 or glucose tolerance. Thus, we set out to determine the acute, *in-vivo* effects of intravenous
115 GIP on i) plasma/serum insulin and NEFA concentrations, and ii) TAG content and gene
116 expression of the key lipid regulating genes, LPL, ATGL and HSL in SAT, in obese
117 individuals with different categories of glucose regulation (normoglycaemic, IGR and
118 T2DM) *versus* lean, normoglycaemic controls.

119

120 **Materials and methods**

121 **Subjects**

122 We studied 23 Caucasian men, age 49 ± 12.3 years (mean \pm SD). Only male subjects were
123 studied to minimise the influence of sex steroids on lipid metabolism (e.g. considering
124 menstrual cycle, menopause or hormone replacement therapy). Subjects with severe cardiac,
125 renal or hepatic disease, endocrine dysfunction, major psychiatric disease, alcohol abuse, and
126 malignancy were excluded. Subjects were sub-divided into four groups according to
127 BMI/glucose regulation: i) lean (n=6), ii) obese (n=6), iii) obese with impaired glucose
128 regulation [obese IGR] (n=6) and iv) obese with (treatment-naive) T2DM [obese T2DM]
129 (n=5).

130

131 Lean and obese were defined according to a BMI ≤ 25 and ≥ 30 kg/m², respectively.
132 Allocation to glucose regulation categories was based on recent medical records combined
133 with a fasting plasma glucose concentration. Obese subjects were allocated to the obese IGR
134 group if they had one/more of the following: fasting hyperglycaemia, impaired glucose
135 tolerance on a 75g oral glucose tolerance test (OGTT) or HbA_{1c} in pre-diabetes range (6-
136 6.5% or 42-47 mmol/mol). Obese subjects with T2DM (according to WHO diagnostic

137 criteria) (40), and not on pharmacological treatment for diabetes were allocated to obese
138 T2DM group. Homeostatic model assessment (HOMA-2) was used to estimate whole body
139 insulin resistance (23); adipose tissue insulin resistance (Adipo-IR) was calculated from
140 fasting NEFA (mmol/L) and insulin (pmol/L) concentration (19). Baseline demographic,
141 anthropometric and biochemical parameters of all participants are shown in Table 1.

142

143 ***Ethical approval*** Ethical approval for this project was obtained from the Northwest Research
144 Ethics Committee, U.K (REC reference **08/H1001/20**). All subjects were studied after
145 informed and written consent.

146

147 **Study protocol**

148 Each subject was studied on two separate occasions, 1-3 weeks apart. After overnight fasting,
149 subjects were infused with either GIP (2 pmol.kg.⁻¹min⁻¹ in 0.9% saline) or placebo (0.9%
150 saline alone). GIP was dosed based on the rate infused in previous studies (16, 35, 38)
151 Subjects were randomly assigned to either GIP/placebo infusion on their initial visit and
152 received the alternate infusion subsequently. Anthropometric assessments were recorded
153 during each visit. Percentage body fat estimation was determined by whole-body
154 bioelectrical impedance analysis (Tanita Corporation, Tokyo, Japan).

155

156 ***GIP infusions, hyperglycaemic clamp and blood sampling*** Intravenous cannulae were
157 inserted into both antecubital fossae, for blood sampling and infusions (GIP/placebo). GIP
158 (Polypeptide Laboratories, Strasbourg, France) was sterile-filtered and dispensed by
159 Stockport Pharmaceuticals (Stepping Hill Hospital, Stockport, U.K). Blood glucose
160 concentration ~8.0 mmol/l was maintained during a hyperglycaemic clamp using priming
161 dose of 20% glucose bolus (based on weight and fasting glucose) given in the first 5 minutes
162 followed by a variable rate infusion of 20% glucose adjusted according to whole blood

163 glucose levels measured every 5 minutes on a YSI blood glucose analyser (YSI U.K Ltd).
164 Intravenous infusion of GIP/placebo was continued from 30 minutes after initiation of
165 hyperglycaemic clamp until 240 minutes. 10 ml blood samples were taken at baseline (prior
166 to hyperglycaemic clamp) and at 15, 30, 60, 120, 180 and 240 minutes following the
167 initiation of GIP/placebo infusion. To minimise protein degradation, aprotinin was added to
168 the tubes prior to sample collection. Samples were centrifuged immediately and serum was
169 stored at -80 degree centigrade until further analysis

170

171 **SAT biopsies** Subcutaneous adipose tissue (SAT) biopsies were obtained at baseline and after
172 240 min of the GIP/placebo infusion on the contralateral site. Under local anaesthesia (1%
173 lidocaine, adrenaline 1:200,000), a small incision was made through the skin and fascia 10cm
174 lateral to the umbilicus. Adipose tissue samples (50-150 mg wet weight) were collected and
175 snap frozen in liquid nitrogen and stored at -80° C until further analysis.

176

177 **Laboratory analysis**

178 **Biochemical analysis** Plasma glucose concentration, lipid profile, liver function parameters
179 and HbA1c were measured using a Cobas 8000 modular analyser (Roche diagnostics, USA).
180 Blood glucose concentrations during hyperglycaemic clamp were measured using YSI 2300
181 STAT glucose analyser (YSI U.K Ltd, Fleet, Hampshire, U.K). Serum insulin was measured
182 by ELISA method (Invitrogen, Fisher Scientific Ltd Loughborough, U.K). Non-Esterified
183 Fatty Acids (NEFAs) were measured from plasma by Randox kit on a Biostat BSD 570
184 analyser (Randox laboratories Ltd, London). Intact GIP was measured at the University of
185 Copenhagen, Denmark: the assay is specific for the intact N-terminus of GIP (biologically
186 active peptide) (13).

187

188 **Subcutaneous Adipose Tissue (SAT) analysis**

189 **SAT lipid content.** Lysates were prepared by homogenization of fat biopsies in a buffer
190 containing: 50mM TrisHCL pH=7.5, 150mM NaCl, 1% Triton X-100, and standard protease
191 inhibitor cocktail (Complete Mini protease inhibitor cocktail, Roche Diagnostics, Germany).
192 Triacylglycerol (TAG) was quantified by measuring free glycerol output following overnight
193 lipase treatment at 37°C (Sigma). The values were normalized according to protein content.

194 **SAT gene expression** Gene expression of LPL, ATGL and HSL were quantified through
195 RNA extraction and real time quantitative PCR. Total RNA was isolated using RNeasy Lipid
196 Tissue Mini Kit (QIAGEN). Real-time quantitative PCR was conducted in triplicate using a
197 BIORAD CFX-connect real time PCR instrument (BioRAD laboratories) using pre-validated
198 TaqMan probes (Life Technologies) as follows: endogenous control β -actin
199 (Hs99999903_m1) and target genes: lipoprotein lipase (lpl, Hs00173425_m1) ATGL
200 (*pnpla2*, Hs00386101_m1), hormone sensitive lipase (lipo. Hs00193510_m1). Relative
201 quantification was carried out using the $\Delta\Delta C_t$ method with β -actin gene expression as an
202 internal control.

203

204 **Statistical analysis**

205 Participant demographics, baseline biochemical parameters and blood glucose concentrations
206 during hyperglycaemic clamp are expressed as mean \pm SD; all other results are expressed as
207 mean \pm SEM. One-way analysis of variance (ANOVA) and Tukey's t- tests were performed
208 to compare participant demographics and baseline biochemical parameters between the four
209 groups in this study. Area under the curve for insulin and NEFA concentrations over 4 hour
210 period of infusion (AUC_{0-4hr}) were calculated by trapezoidal rule using GraphPad Prism
211 software. Paired t-tests were performed on changes in gene expression and lipid content
212 (SAT-TAG) parameters to explore whether the change over the two time points differed

213 between GIP and placebo. P value of < 0.05 (two-tailed) was considered to be significant.
214 A Pearson product-moment correlation coefficient was computed to assess the relationship
215 between degree of NEFA reduction and other variables (fasting plasma glucose and Adipose
216 tissue insulin resistance (Adipo-IR).
217 A linear mixed-effects model was also used to model insulin secretion and NEFA
218 concentrations using three time points (baseline, 120 minutes and 240 minutes). Main effects
219 for the four different groups are included along with a two-way interaction between treatment
220 and group. This allows that the overall effect of GIP infusion in comparison to the placebo
221 infusion can be assessed individually for different groups. Results are expressed in estimated
222 average unit changes in insulin and NEFAs during GIP vs. placebo infusion.

223

224 **Results**

225 **Baseline characteristics (Table 1)**

226 *Patient demographics*

227 Twenty three individuals completed the study protocol in four sub-groups: lean (n=6), obese
228 (n=6), obese IGR (n=6) and obese T2DM (n=5). Waist circumference and percentage body
229 fat mass were significantly higher in obese, obese IGR, obese T2DM compared to the lean
230 group. The duration of diabetes in obese T2DM group was 7 ± 5.5 months (mean \pm SD),
231 mean HbA1c of 54 ± 8.5 mmol/mol (7.1 ± 0.8 %) and all participants were naive to oral or
232 injectable diabetes medications.

233

234 *Baseline biochemistry*

235 *Plasma glucose and insulin concentrations*

236 As expected, mean fasting glucose was higher in obese IGR and obese T2DM groups
237 compared to the two other groups. Fasting insulin and HOMA-IR were significantly higher in

238 obese, obese IGR and obese T2DM groups vs. the lean group. Adipo-IR was significantly
239 higher in obese T2DM group vs. lean and obese groups but not vs. obese IGR group (Table 1)

240

241 ***Metabolic parameters***

242 All subjects in obese IGR and obese T2DM groups had metabolic syndrome based on
243 International Diabetes Federation 2006 criteria (2) with most consequently treated for
244 hypertension and dyslipidemia: ACE inhibitors or angiotensin receptor blockers (three
245 subjects in obese IGR group, five subjects in obese T2DM group), beta-blockers (two obese
246 IGR, 2 obese T2DM) and calcium channel blocker (one obese T2DM). Three subjects in each
247 of the above two groups were on statins. Two subjects in the obese group had metabolic
248 syndrome (one on ACE inhibitors and one a fibrate). [Table 1].

249

250 **Biochemistry changes during infusions**

251 ***Blood glucose.*** The blood glucose concentrations were maintained at ~8.0 mmol/l during the
252 hyperglycaemic clamp with both GIP and placebo infusions in all four groups (Figure 1A-D).
253 The whole blood glucose concentrations (mean \pm SEM) from measurements at 15 minute
254 intervals during 4 hour hyperglycaemic clamp in the four groups were: lean, 8.02 ± 0.02
255 (GIP) vs. 8.17 ± 0.14 mmol/l (placebo); obese, 8.0 ± 0.07 (GIP) vs. 8.17 ± 0.07 mmol/l
256 (placebo); obese IGR group, 8.08 ± 0.11 (GIP) vs. 8.11 ± 0.06 mmol/l (placebo) in and obese
257 T2DM group, 8.35 ± 0.15 (GIP) vs. 8.46 ± 0.18 mmol/l (placebo).

258 The volume of 20% glucose (mean \pm SEM) infused to maintain the hyperglycaemic clamp
259 during GIP vs. placebo infusions in the four groups were: lean, 1124 ± 155 mls (GIP) vs.
260 631 ± 152 mls (placebo); obese, 926 ± 150 (GIP) vs. 462 ± 106 mls (placebo) obese IGR
261 group, 725 ± 139 (GIP) vs. 398 ± 34 mmol/l (placebo) in and obese T2DM group, 508 ± 72
262 (GIP) vs. 323 ± 14 mls (placebo).

263 **Plasma GIP** Fasting plasma GIP concentrations were similar across the four groups for both
264 visits with higher GIP concentrations achieved during GIP infusions. Plasma GIP (mean \pm
265 SEM) at baseline, 120 and 240 minutes in the four groups are as follows: lean (12.8 ± 1.1 ,
266 30.5 ± 4.6 , 23.2 ± 2.6 pmol/l with GIP vs. 13.7 ± 2.2 , 8.3 ± 1.9 , 9.7 ± 2.8 pmol/l with
267 placebo, obese (15.2 ± 2.9 , 38.8 ± 6.9 , 21.8 ± 5.3 pmol/l with GIP vs. 13.0 ± 2 , 15 ± 3.4 , 15.2
268 ± 5 pmol/l with placebo), obese IGR (14.2 ± 3.7 , 38.2 ± 7 , 26.7 ± 4.7 pmol/l with GIP vs.
269 12.2 ± 2.9 , 13.5 ± 2.5 , 12.8 ± 1.6 pmol/l with placebo), obese T2DM (14.2 ± 2 , 51.6 ± 7.2 , 26
270 ± 7.2 pmol/l with GIP vs. 14.4 ± 2 , 23 ± 9.8 , 17.8 ± 6.5 pmol/l with placebo).

271

272 **Serum insulin** The insulin concentrations (mean \pm SEM) during GIP and placebo infusions
273 along with hyperglycaemic clamp are shown in Figure 2 A-D. Mean AUC_{0-4hr} of insulin
274 concentrations (μ IU/ml/min) was higher with GIP infusion compared to placebo in the
275 following groups: Lean (49317 ± 6009 vs. 22670 ± 4361 ; $p= 0.01$), obese (71956 ± 8860 vs.
276 45921 ± 10065 ; $p=0.1$) and obese IGR groups (61884 ± 6653 vs. 20061 ± 3140 ; $p=0.001$)
277 respectively. In T2DM group, the AUC_{0-4hr} of insulin during GIP infusion was not different
278 from placebo (25151 ± 4103 vs. 20913 ± 5514 ; $p= 0.28$) [Figure 2 E].

279 The change in insulin concentration over 240 minutes, compared to baseline values, differed
280 by 63, 70 and 121 μ IU/ml with GIP infusion vs. placebo in lean, obese and obese IGR groups
281 respectively. In obese T2DM group, there was only a 9 μ IU/ml increase in insulin
282 concentration with GIP vs. placebo infusion (Figure 2F)

283

284 **Plasma Non-Esterified Fatty Acids (NEFAs)** Circulating NEFAs (mean \pm SEM) reduced
285 from baseline during both GIP and placebo infusions in all four groups under hyperglycaemic
286 clamp conditions (Figure 3A-D). Mean AUC_{0-4hr} for NEFAs were not different with GIP vs.
287 placebo in lean and obese groups (15234 ± 1610 vs. 15520 ± 1884 ; $p= 0.9$ in lean group and

288 22345 ± 4644 vs. 28770 ± 6057; p= 0.42 in obese group respectively) [Figure 3E]. NEFAs in
289 obese IGR group appear to be lower with GIP (Figure 3C), but the mean AUC_{0-4hr} (21119 ±
290 1882 vs. 32573 ± 3638; p=0.055; 95% CI 0.42 to 1.01) and reductions on a linear mixed
291 model were not statistically significant (Figure 3 E, F). Whereas in obese T2DM group the
292 mean AUC_{0-4hr} of NEFAs (μmol/L/min) was significantly lower with GIP infusion compared
293 to placebo (41992 ± 9843 vs. 71468 ± 13605; p= 0.039; 95% CI 0.31 to 0.95) and
294 there was 82.6 μmol/L reduction in NEFAs from baseline to 240 minutes with GIP infusion
295 compared to placebo (95% CI, -139, -26; p = 0.004) [Figure 3 E, F].

296 The degree of reduction in NEFA (ΔNEFA) with GIP infusion across all subjects (n=23)
297 correlated positively with fasting plasma glucose (Pearson r = 0.44, p = 0.03) and Adipo-IR
298 (Pearson r = 0.56, p = 0.005) (Figure 4).

299

300 **Serum triacylglycerol concentration** There were no significant alterations in serum
301 triacylglycerol (TAG) concentrations with either GIP or placebo in any of the four groups
302 (data not shown).

303

304 **Subcutaneous Adipose Tissue (SAT) changes**

305 **SAT triacylglycerol (TAG) content** The changes in lipid content after 240 minutes of GIP vs.
306 placebo infusion relative to respective baselines on each visit are shown in Figure 5. In the
307 obese T2DM group, the SAT-TAG content increased 1.78 ± 0.4 fold (mean ± SEM) from
308 baseline with GIP infusion compared to 0.86 ± 0.1 fold with placebo (95% CI:0.1,1.8;
309 p=0.043). The changes in TAG content in the other three groups were not statistically
310 significant (data shown in Figure 5)

311 **Gene expression of enzymes involved in lipid metabolism.** The changes in mRNA
312 expression (LPL, ATGL and HSL) in SAT after 240 minutes of GIP vs. placebo infusion
313 relative to respective baselines on each visit are shown in Figure 6.

314

315 **LPL**, The LPL mRNA expression in the T2DM group was 1.25 fold higher from baseline
316 with GIP infusion compared to 0.94 fold change with placebo but this was not statistically
317 significant (p=0.27). In the other three groups the changes in LPL mRNA expression with
318 GIP and placebo were comparable (Figure 6A).

319

320 **ATGL** In the T2DM group, ATGL mRNA expression was higher with GIP infusion
321 compared to placebo (1.5 vs. 1.1 fold; p=0.12) but this was not statistically significant. In the
322 other three groups the changes in ATGL gene expression with GIP versus placebo were
323 comparable (Figure 6B).

324

325 **HSL** The changes in HSL gene expression with GIP did not differ significantly compared to
326 placebo in all four groups (Figure 6C). Fold change data for the three enzymes in all four
327 groups is shown in Figure 6D.

328

329 **Discussion**

330 We demonstrate that acute GIP infusion, during fasting, under hyperglycaemic conditions,
331 reduced serum/plasma NEFAs, concomitantly increasing SAT triacylglycerol (TAG) content
332 in obese patients with T2DM. This anabolic effect was not observed in the lean, obese or
333 obese patients with IGR. In contrast, while GIP was able to stimulate insulin secretion in the
334 lean, obese or obese patients with IGR, its insulinotropic action was not observed in obese
335 patients with T2DM. Thus, in obese patients with T2DM, there is a dissociation of the effects

336 on GIP on beta cells and adipocytes, with blunted insulinotropic but preserved lipogenic
337 actions respectively.

338

339 Expression of the GIP receptor (GIPR) is somehow glucose dependent and down regulated in
340 response to hyperglycaemia (24). In patients with T2DM the blunted incretin effect
341 (involving both incretin hormones, GLP-1 and GIP) may in part be due to reduced islet cell
342 expression of GIP receptors (GIPR) secondary to chronic hyperglycemia (16, 29, 35, 39).
343 The physiological role of GIP in adipose tissue in T2DM remains unclear although adipose
344 GIPR expression may be similarly down regulated in insulin resistant human subjects and
345 may represent a compensatory mechanism to reduce fat storage in insulin resistance,
346 considering the interference of NEFAs on insulin signal transduction (10, 22). However,
347 energy dense, high fat diets in obese individuals with T2DM could result in exaggerated fat
348 storage (through exaggerated GIP release) even in the absence of adequate insulin secretion.
349 Although we did not measure GIPR, the lipogenic action of GIP at the adipocyte appears to
350 be more pronounced in T2DM (Figure 5). Studies in patients with NAFLD suggests elevated
351 GIP secretion is also associated with intra-hepatocellular lipid deposition (33).

352

353 Several factors may explain the differential ability of GIP to increase NEFA re-esterification
354 in SAT in obese T2DM subjects versus other groups. In lean, obese and obese individuals
355 with IGR, where insulin secretion is potently stimulated and adipose tissue insulin sensitivity
356 is preserved (lower Adipo-IR), insulin independently suppressed lipolysis, lowering NEFAs
357 perhaps leaving GIP's effects trivial. However, in T2DM when insulin secretion is impaired
358 and adipose tissue is insulin resistant (high Adipo-IR), the effect of GIP assumes greater
359 importance, promoting lipid accumulation in adipocytes. This is consistent with animal data.
360 GIP does not promote fat accumulation in adipocytes with normal insulin sensitivity, with

361 GIPR^{-/-} mice showing similar adiposity to wild-type on control diet (31). However, under
362 conditions of diminished insulin action, using IRS1 deficient mice, when the effects of GIP
363 are examined (by disrupting GIP signaling, GIP^{-/-} vs. GIPR^{+/+}) GIP was shown to promote
364 SAT and VAT expansion and decrease fat oxidation with greater SAT and VAT mass and
365 lower fat oxidation in IRS-1^{-/-}GIPR^{-/-} vs. IRS-1^{-/-}GIPR^{+/+} mice (42).

366

367 A few human studies examined the metabolic effect of an acute GIP infusion in lean and
368 obese individuals but none reported in people with T2DM. In studies to date, the effects of
369 GIP have been examined under different experimental conditions to those here, for example
370 during concomitant intralipid infusion and/or with hyperinsulinaemic-hyperglycaemic clamp
371 conditions and measuring arteriovenous concentrations of metabolites. These data
372 demonstrated that in lean people, GIP in combination with hyperinsulinaemia and
373 hyperglycemia, increased adipose tissue blood flow, glucose uptake, and FFA re-
374 esterification, thus resulting in increased abdominal SAT-TAG deposition (4-6). The same
375 group showed that in obese and IGR subjects GIP infusion did not have the same effect on
376 adipose tissue blood flow or TAG deposition in adipose tissue (3). However, the independent
377 contributions of insulin vs. GIP to these metabolic effects are difficult to dissect although GIP
378 *per se* appeared to have little effect on human subcutaneous adipose tissue in lean insulin
379 sensitive subjects, with an effect only apparent when GIP was co-administered with insulin
380 during hyperglycemia. Thus it would appear that there are direct and indirect effects of GIP.

381

382 During nutrient excess, lipogenesis is stimulated via lipoprotein lipase (LPL), hydrolysing
383 circulating lipoprotein-derived triglycerides and promoting NEFA esterification into TAG
384 and storage within lipid droplets of adipose tissue. During periods of fasting, mobilisation of
385 NEFAs from fat depots relies on the activity of key hydrolases, including hormone-sensitive

386 lipase (HSL) and adipose triglyceride lipase (ATGL). In SAT, insulin stimulates NEFA
387 esterification by enhancing lipoprotein lipase (LPL), and inhibits lipolytic process (18). The
388 majority of the animal studies have shown that GIP potentiates the role of insulin in
389 regulation of LPL, and NEFA incorporation into adipose tissue (9, 15, 27, 31). GIP enhanced
390 LPL gene expression in cultured subcutaneous human adipocytes through pathways involving
391 protein kinase B and AMP-activated protein kinase (26, 28). Trying to determine the
392 molecular mechanism by which SAT-TAG content changed, we measured SAT mRNA
393 expression of LPL, ATGL and HSL; surprisingly, we observed no significant changes in
394 expression to account for altered serum NEFAs or SAT-TAG content. This may represent a
395 time-course phenomenon (changes in gene expression with GIP in human adipose tissue may
396 occur over a longer interval). This speculation is consistent with the slow temporal onset of
397 the molecular responses in adipose tissue in animal studies. GIP infusion may affect enzyme
398 activity rather than gene expression and therefore results may differ if
399 activity/phosphorylation was measured. To better appreciate the physiological effects of GIP
400 administration on human SAT, stable isotope studies to determine dynamic changes in fat
401 metabolism with serial tissue biopsies are required.

402

403 All studies were performed under hyperglycaemic clamp conditions to achieve comparable
404 hyperglycaemia and to mimic post-prandial increases in GIP and insulin. The peak GIP
405 concentrations achieved in our study during GIP infusions were comparable to levels
406 achieved elsewhere (3). We believe the changes in NEFAs and SAT lipid content in our
407 obese T2DM are more likely due to the effect of GIP, particularly in the absence of excess
408 insulin secretion. Reductions in NEFA correlated positively with fasting glucose and
409 Adipo-IR in all the subjects across the four groups suggesting the effects of GIP are more
410 pronounced in hyperglycaemic and insulin resistant states. We recognise that higher Δ NEFA

411 would be expected in subjects with higher fasting NEFA levels however correlation with
412 Adipo-IR was only seen with GIP but not with placebo infusion (Figure 4).

413

414 Studying four distinct groups (with differing BMI and glucose tolerance) facilitates
415 evaluation of the differential effects of GIP in insulin sensitive and resistant individuals.

416 However, we acknowledge limitations including small group sizes and the degree of obesity:

417 there was limited pilot data in humans prior to initiation of this study and subsequently

418 published human studies on GIP infusion had small number of subjects (3-5). Findings from

419 our study may differ in less severely obese individuals. Lean subjects were younger

420 compared to others and may have increased insulinotropic activity to GIP (30) but there was

421 no significant difference in Insulin AUC between the groups except in obese T2DM.

422 Unrecognised interactions between anti-hypertensive or lipid modifying medication and

423 effects of GIP cannot be excluded.

424

425 In conclusion, we demonstrate that in obese patients with T2DM, acute GIP infusion in a

426 fasting state, during hyperglycaemia, lowers serum NEFA and increases the SAT lipid

427 content despite reduced insulinotropic activity. In lean, obese and obese with IGR, despite the

428 intact insulinotropic response to GIP no lipogenic effect was observed. This anabolic effect of

429 GIP further exacerbates obesity and insulin resistance.

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436

437

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444

445

446 **Disclosure summary**

447 None of the authors have a conflict of interest in relation to this submitted work. Professor
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591 **Figure legends**

592

593 **Figure 1:** Study protocol showing the duration of hyperglycaemic clamp and the time point
594 for the start of GIP / placebo infusions. The of blood glucose concentrations (Mean \pm SEM)
595 at 15 minute intervals for the duration of hyperglycemic clamp during placebo and GIP visits
596 are shown in **A** lean individuals, **B** obese, individuals, **C** obese individuals with IGR, **D** obese
597 individuals with T2DM.

598

599 **Figure 2:** Serum insulin concentrations (mean \pm SEM) during 4 hour infusions of GIP vs.
600 placebo (with hyperglycaemic clamp) are shown in **A** lean individuals, **B** obese, individuals,
601 **C** obese individuals with IGR, **D** obese individuals with T2DM. The time points for baseline
602 blood sampling* and start of GIP/placebo infusions are shown on the X axis. **E** AUC_{0-4hr} for
603 insulin concentrations during the 4-hour infusion of GIP versus placebo for the above four
604 groups (p values: *0.01; ** 0.001). **F** Linear mixed model analysis showing the increase in
605 insulin concentrations with GIP compared to placebo infusion over 240 minutes, confidence
606 intervals (CI) and p values

607

608 **Figure 3:** Plasma NEFA concentrations (mean \pm SEM), during 4 hour infusions of GIP vs.
609 placebo (with hyperglycaemic clamp) are shown in **A** lean individuals, **B** obese individuals,
610 **C** obese individuals with IGR, **D** obese individuals with T2DM. The time points for baseline
611 blood sampling* and start of GIP/placebo infusions are shown on the X axis. **E** AUC_{0-4hr} for
612 NEFA concentrations during the 4-hour infusion of GIP versus placebo for the above four
613 groups (p values: * <0.05). **F** Linear mixed model analysis showing the decrease in NEFA
614 concentrations with GIP compared to placebo infusion over 240 minutes, confidence
615 intervals (CI) and p values.

616

617 **Figure 4:** **A, B** The correlation between plasma fasting glucose and changes in NEFA at 240
618 minutes from baseline (Δ NEFA_{0-240 min}) during placebo and GIP infusions. **C, D** The
619 correlation between Adipo-IR and changes in NEFA at 240 minutes from baseline
620 (Δ NEFA_{0-240 min}) during placebo and GIP infusions. Pearson's r is represented as r and p
621 value (two tailed) with statistical significance * (<0.05) and ** (<0.01)

622

623

624 **Figure 5:** **A** Fold changes (mean \pm SEM) in subcutaneous adipose tissue (SAT)
625 triacylglycerol (TAG) content after 240 min GIP vs. placebo infusion relative to the baseline
626 on the same day in lean individuals, obese individuals, obese individuals with IGR and obese
627 individuals with T2DM. **B** Fold change values, confidence intervals (CI) and p values

628

629 **Figure 6:** Fold changes (mean \pm SEM) in SAT gene expression of **A** LPL **B** ATGL and **C**
630 HSL after 240 min of GIP vs. placebo infusion relative to baseline on the same day in lean
631 individuals, obese individuals, obese individuals with IGR and obese individuals with T2DM,
632 **D** Fold change values, confidence intervals (CI) and p values.

633

634 **Figure 7:** In healthy people, GIP acts on its receptors on beta cells and adipocytes to promote
635 insulin secretion (insulinotropic action) and lipid deposition (adipogenic action) (*left figure*).
636 In obesity, with consumption of an energy-dense, higher fat diet, there is enhanced insulin
637 secretion (which may help overcome peripheral insulin resistance) and increased lipid
638 deposition (which will further enhance fat storage) (*middle figure*). In T2DM, the effects of
639 GIP on beta cell are impaired with reduced insulin secretion; the effects on the adipocyte
640 seem to be preserved further promoting lipid deposition (*right figure*).

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Table 1 Baseline demographic, anthropometric and biochemical parameters (mean \pm SD).

	Lean (N=6)	Obese (N=6)	Obese IGR (N=6)	Obese T2DM (N=5)
Age (years)	35 \pm 7	47 \pm 12	57 \pm 8*	57 \pm 8 *
BMI (kg/m ²)	24 \pm 1	40 \pm 8**	37 \pm 5*	45 \pm 13***
Waist Circumference (cm)	94 \pm 5	129 \pm 19**	124 \pm 14**	140 \pm 17***
Body fat mass (%)	18 \pm 3	38 \pm 6****	31 \pm 16****	46 \pm 6****
Systolic BP (mmHg)	131 \pm 15	136 \pm 14	141 \pm 3	135 \pm 12
Diastolic BP (mmHg)	78 \pm 8	73 \pm 5	72 \pm 6	76 \pm 14
Alanine transaminase (U/L)	21 \pm 6	27 \pm 21	30 \pm 17	24 \pm 11
Fasting cholesterol (mmol/l)	5.2 \pm 0.7	5.0 \pm 0.3	3.9 \pm 0.6*	4.3 \pm 1.0
HDL (mmol/L)	1.3 \pm 0.3	1.1 \pm 0.1	0.9 \pm 0.2*	0.8 \pm 0.1*
LDL (mmol/L)	3.4 \pm 0.9	3.2 \pm 0.5	2.5 \pm 0.8	2.8 \pm 0.9
Triglycerides (mmol/l)	1.1 \pm 0.1	1.5 \pm 0.3	1.9 \pm 1.5	1.5 \pm 0.5
Fasting plasma glucose (mmol/l)	5.3 \pm 0.3	5.1 \pm 0.9	6.0 \pm 0.7	6.8 \pm 1.1* ^Δ
Fasting Insulin (μ IU/ml)	11.9 \pm 2.6	30.5 \pm 14.4*	38.3 \pm 12.5**	36.9 \pm 9.1**
Fasting NEFAs [‡] (μ mol/L)	352 \pm 118	312 \pm 123	421 \pm 115	494 \pm 150
HOMA-IR [¥]	1.6 \pm 0.3	3.8 \pm 1.8*	4.8 \pm 1.4**	4.9 \pm 1.2**
Adipo-IR [§] (mmol/L/pmole/L)	24.5 \pm 8.1	54 \pm 23.7	95.9 \pm 37.8**	115.7 \pm 51.2** ^Δ
HbA1c (mmol/mol)	-	-	44 \pm 2.3	54 \pm 8.5

P value for statistically significant difference vs. Lean group is indicated as * (<0.05); ** (<0.01); *** (<0.001); **** (<0.0001) and p value for significant difference vs. obese group is indicated as ^Δ (<0.05). [‡] Non Esterified Fatty Acids (NEFA), [¥] Homeostasis Model Assessment-Insulin resistance (HOMA-IR), [§] Adipose tissue insulin resistance (Adipo-IR)

Figure 1

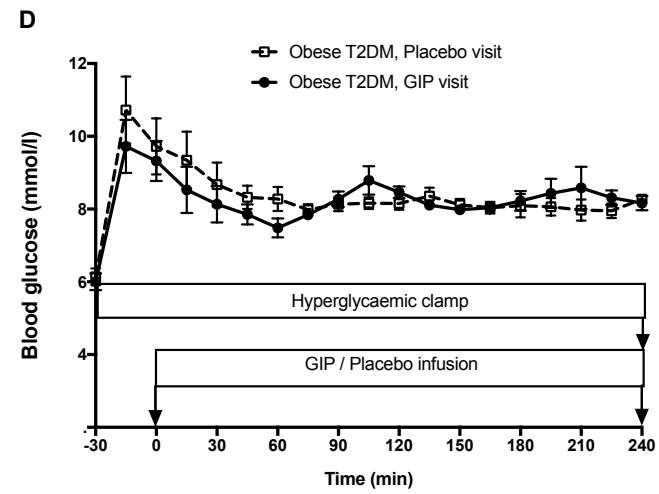
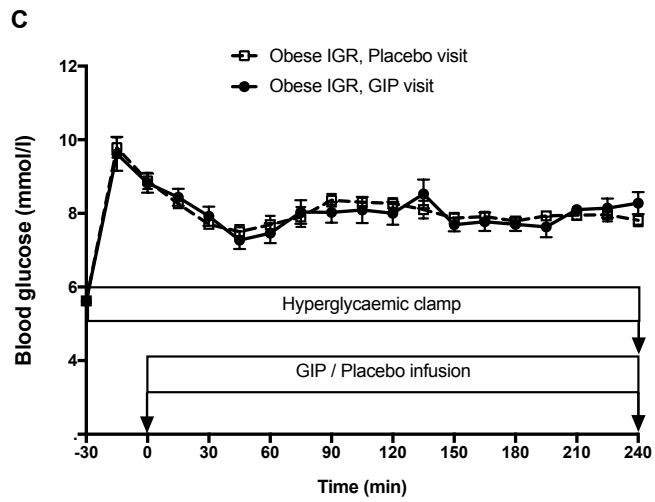
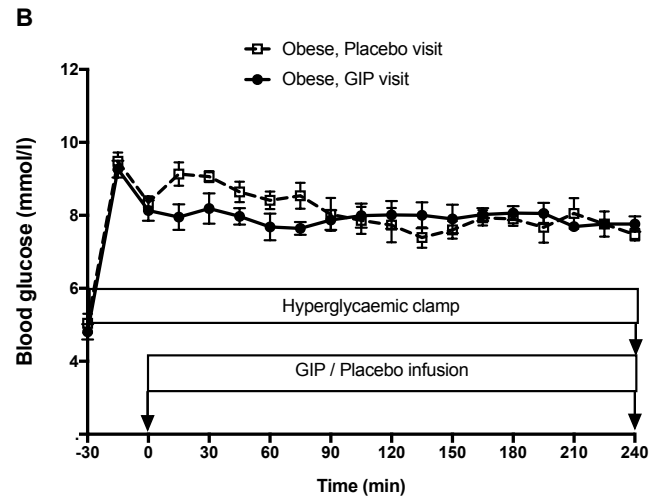
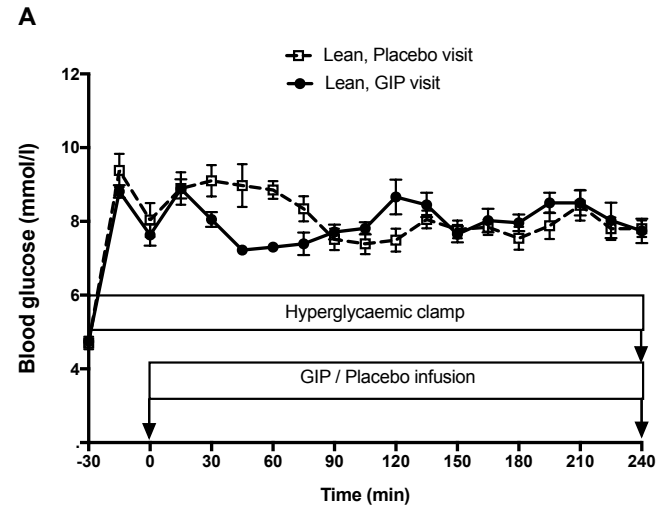
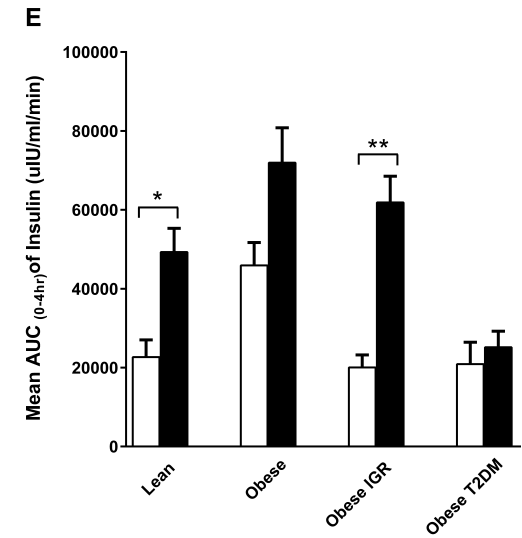
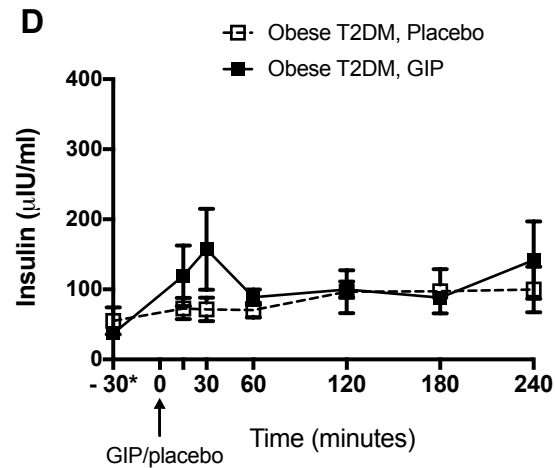
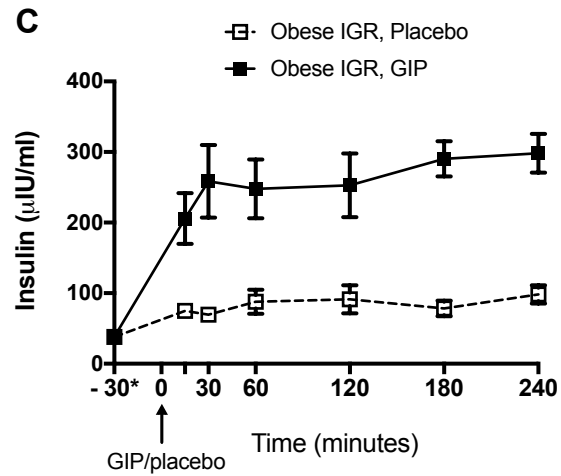
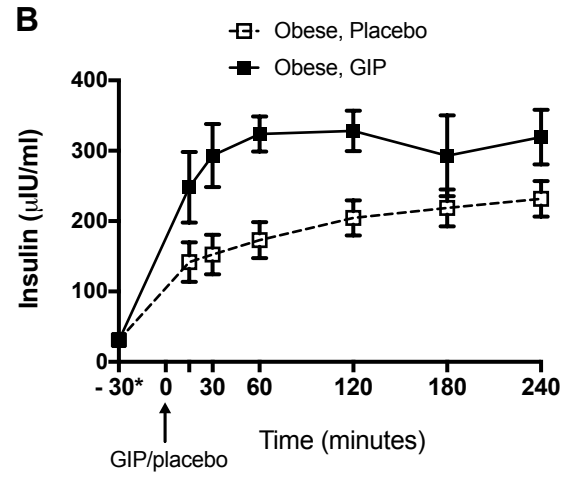
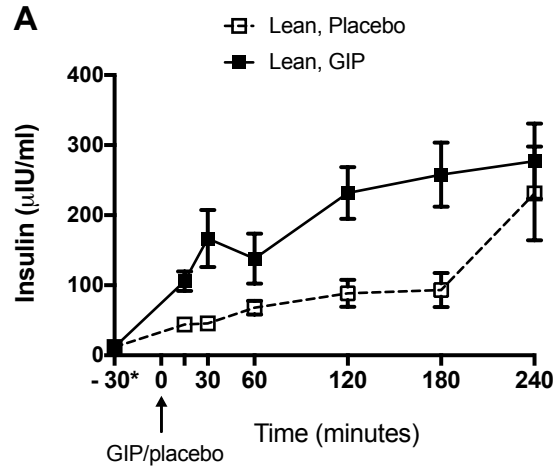


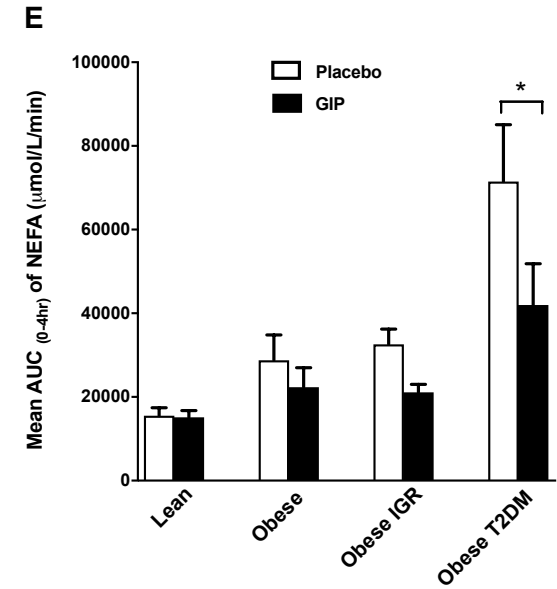
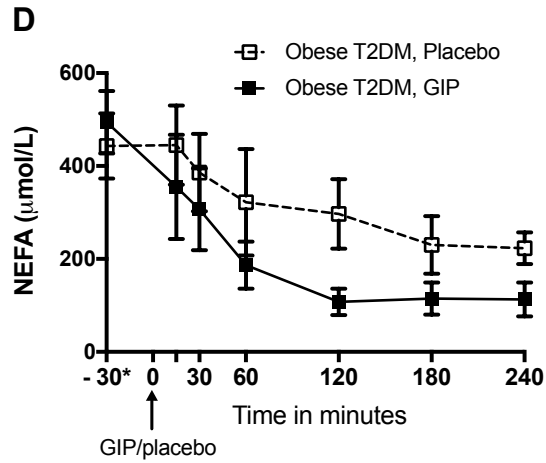
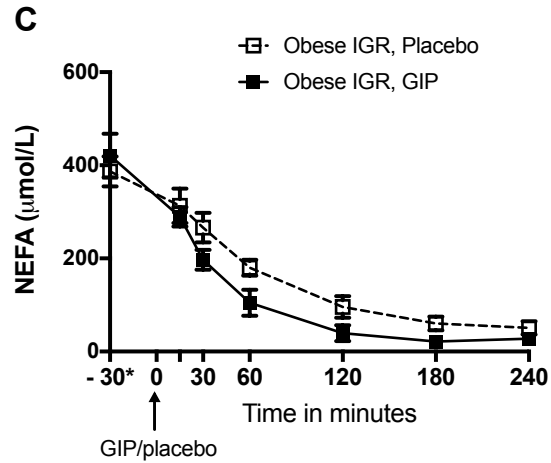
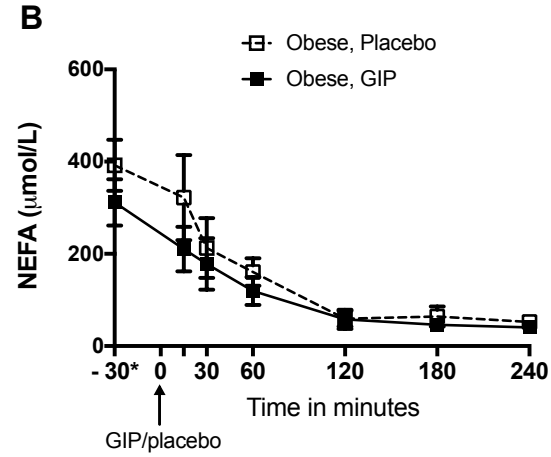
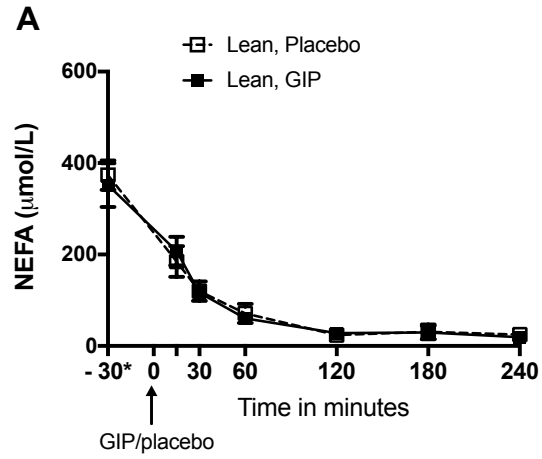
Figure 2



F

	Increase in insulin concentration (μIU/ml)	95% CI	p-value
GIP vs. placebo			
Lean	63	(10, 115)	0.019
Obese	70	(18, 12)	0.009
Obese IGR	121	(68, 173)	<0.001
Obese T2DM	9	(- 49, 67)	0.76

Figure 3



F

	Decrease in NEFA Concentrations ($\mu\text{mol/l}$)	95% CI	p value
GIP vs. Placebo			
Lean	7.9	(-59, 44)	0.763
Obese	31.2	(-82, 20)	0.234
Obese IGR	11.4	(-63., 41)	0.668
Obese T2DM	82.6	(-139, -26)	0.004

Figure 4

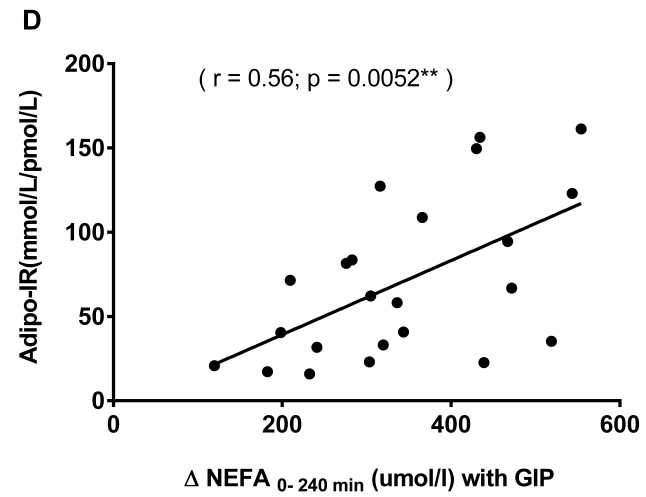
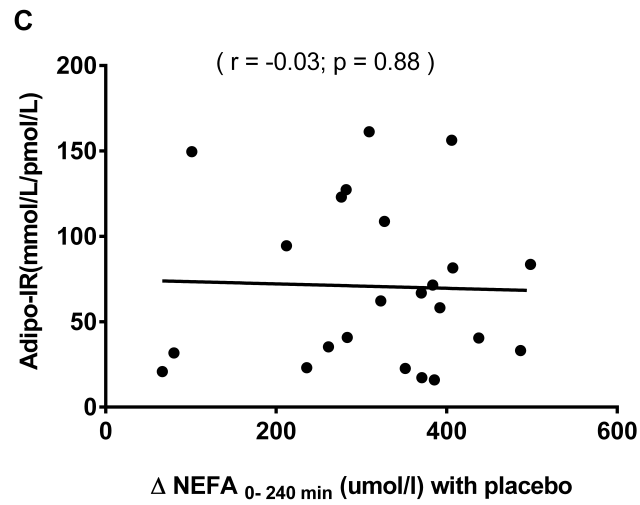
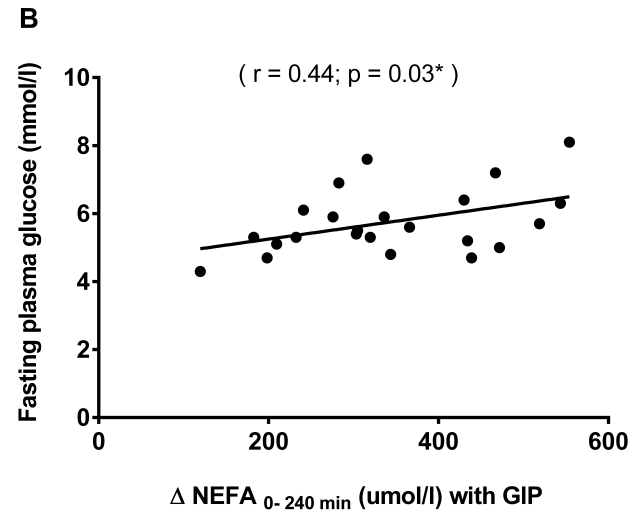
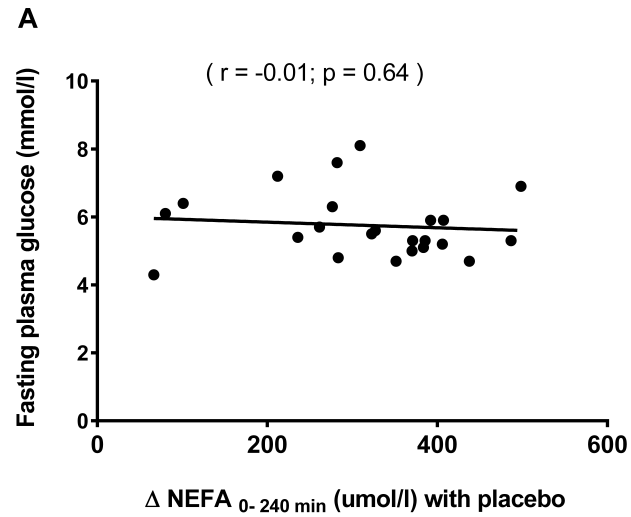
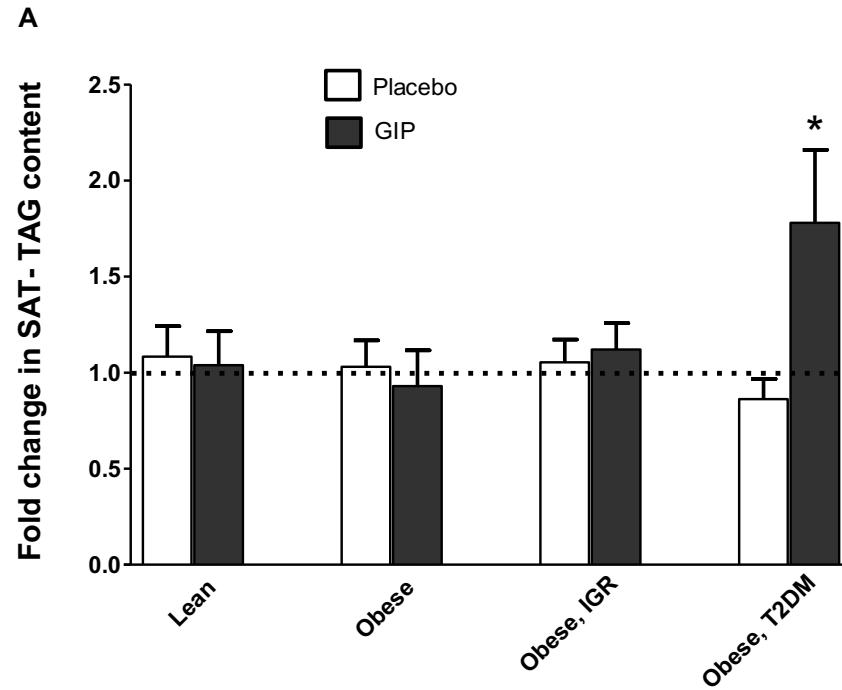


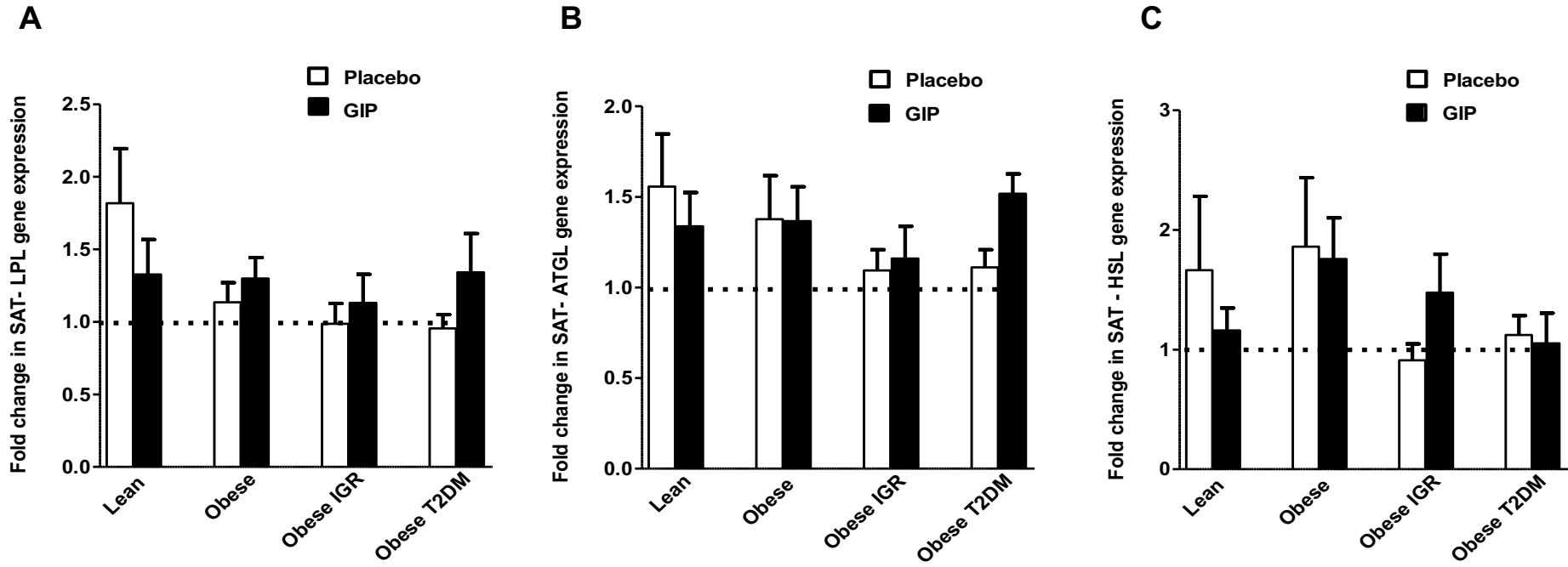
Figure 5



B

Groups	Fold change (mean ± SEM) in SAT-TAG content relative to baseline		95% CI	p-value
	Placebo	GIP		
Lean	1.08 ± 0.16	1.03 ± 0.18	(-0.5, 0.6)	0.84
Obese	1.03 ± 0.14	0.93 ± 0.19	(-0.43,0.62)	0.65
Obese IGR	1.05 ± 0.12	1.12 ± 0.14	(-0.56,0.4)	0.73
Obese T2DM	0.86 ± 0.1	1.78 ± 0.38	(0.1,1.8)	0.043*

Figure 6



D

Fold change (mean ± SEM) in SAT gene expression relative to respective baselines on each visit									
Groups	LPL			ATGL			HSL		
	Placebo	GIP	P value	Placebo	GIP	P value	Placebo	GIP	P value
Lean	1.8 ± 0.4	1.2 ± 0.2	0.38	1.6 ± 0.3	1.3 ± 0.2	0.71	1.7 ± 0.6	1.2 ± 0.2	0.42
Obese	1.2 ± 0.1	1.3 ± 0.1	0.49	1.3 ± 0.2	1.3 ± 0.2	0.96	1.9 ± 0.6	1.8 ± 0.3	0.93
Obese IGR	0.9 ± 0.1	1.1 ± 0.2	0.64	1.1 ± 0.1	1.2 ± 0.2	0.90	0.9 ± 0.1	1.5 ± 0.3	0.16
Obese T2DM	0.9 ± 0.1	1.4 ± 0.2	0.27	1.1 ± 0.1	1.5 ± 0.1	0.12	1.1 ± 0.2	1.0 ± 0.2	0.62

Figure 7

