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The endocrine disrupting potential of monosodium glutamate (MSG) on secretion of the glucagon-like peptide-1 (GLP-1) gut hormone and GLP-1 receptor interaction

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24 **Abstract**

25 Monosodium glutamate (MSG) is a suspected obesogen with epidemiological evidence
26 positively correlating consumption to increased body mass index and higher prevalence of
27 metabolic syndrome.

28 ELISA and high content analysis (HCA) were employed to examine the disruptive effects of
29 MSG on the secretion of enteroendocrine hormone glucagon-like peptide-1 (GLP-1) and
30 GLP-1 receptor (GLP-1R), respectively. Following 3 h MSG exposure of the enteroendocrine
31 pGIP/neo: STC-1 cell line model (500 µg/ml) significantly increased GLP-1 secretion (1.8
32 fold; $P<0.001$), however, 72 h exposure (500 µg/ml) caused a 1.8 fold decline ($P<0.05$).
33 Also,3 h MSG exposure (0.5-500 µg/ml) did not induce any cytotoxicity (including multiple
34 pre-lethal markers) but 72 h exposure at 250-500 µg/ml, decreased cell number (11.8-26.7%;
35 $P<0.05$), increased nuclear area (23.9-29.8%; $P<0.001$) and decreased mitochondrial
36 membrane potential (13-21.6%; $P<0.05$). At 500 µg/ml, MSG increased mitochondrial mass
37 by 16.3% ($P<0.01$). MSG did not agonise or antagonise internalization of the GLP-1R
38 expressed recombinantly in U2OS cells, following GLP-1 stimulation. In conclusion, 72 h
39 exposure of an enteroendocrine cell line at dietary levels of MSG, results in pre-lethal
40 cytotoxicity and decline in GLP-1 secretion. These adverse events may play a role in the
41 pathogenesis of obesity as outlined in the *obesogen hypothesis* by impairing GLP-1 secretion,
42 related satiety responses and glucose-stimulated insulin release.

43 ***Key terms:*** Monosodium glutamate, *in vitro* bioassays, high content analysis, GLP-1,
44 diabetes; obesity

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51 **1. Introduction**

52 The incidence of obesity and metabolic diseases such as diabetes has risen dramatically over
53 the past two decades. The United Kingdom (UK) is officially the most obese country in
54 Europe with 1 in 5 adults overweight and 1 in 15 adults obese. It is predicted that the number
55 of obese individuals will soar by a staggering 73% to 26 million people in the UK over the
56 next twenty years. This is expected to fuel increases in cases of diabetes which in 2010 were
57 estimated at 285 million worldwide with a predicted increase by 54% in 2030 due to
58 population growth, ageing of populations and urbanization with associated lifestyle change
59 (Shaw *et al.,* 2010). Increased caloric intake and decreased physical activity are undoubtedly
60 the major drivers of these increases but other factors have been highlighted (COT, 2013).
61 Several lines of evidence, including several human epidemiological studies, suggest that
62 certain chemicals in the diet or environment (referred to as 'obesogens') play a role in
63 promoting obesity (Sharpe and Drake, 2013).

64 Monosodium glutamate (MSG) is a flavour enhancer used in many household prepared and
65 processed foods worldwide which has recently been highlighted as a suspected dietary
66 obesogen (Holtcamp, 2012). It is also believed that the effects of MSG are compounded
67 further by its widespread availability in manufactured foods and the high frequency with
68 which some individuals consume it (Mercola, 2009). A study investigating the effect of a
69 lipid-containing meal with added MSG (2000 mg/meal) on glucose homeostasis, incretin
70 secretion and gastric emptying in humans demonstrated GLP-1 plasma levels were
71 significantly increased (Hosaka et al., 2012).. This demonstrates that exposure to MSG can
72 have effects on the regulation of gut hormone secretion and consequently, glucose and weight
73 homeostasis and highlights its potential to act as an endocrine disruptor.

74 The reported daily MSG intake of individuals varies greatly across the population. The
75 reported UK average intake in 1991 was 580 mg/day for the general population but 4.68
76 g/day for excessive consumers. In 2000, the average intake ranged from 300-1000 mg/day in
77 industrialised countries (Husarova and Ostatnikova, 2013). However, more recent studies
78 state that the average intake of MSG is around 0.4 g/day in Europe and has been steadily
79 increasing (Collision *et al.,* 2009, He *et al.,* 2011, Nakanishi *et al.,* 2008). It is also important
80 to consider Asian countries, where MSG consumption is much higher than other parts of the
81 world. A study conducted in China found that the mean intake of MSG for the entire
82 population was 3.1 g/ day (Shi *et al.,* 2014). Current legislation does not impose any limit on
83 the amount of MSG that restaurants or the food industry can add to their products.
84 Furthermore, food processors and manufacturers are not obligated to list the amount of MSG

on their packaging. This anomaly makes it difficult to accurately monitor levels of MSG consumption and underestimations most likely occur.

A number of studies have investigated potential links between MSG, obesity and other metabolic disorders such as diabetes. In a longitudinal study, He *et al.* (2011) examined the association between MSG consumption and the incidence of overweight adults, reporting that a cumulative mean (± standard deviation) MSG intake of 2200 ± 1600 mg/day is associated with an increase in body mass index (BMI) after adjustment for potential confounders. The extent of weight gain in MSG users compared with non-users was modest. However, there is still major public health interest in knowing whether weight gain is modulated by MSG intake. Further studies are necessary to investigate potential mechanisms where MSG could enhance the risk of obesity/diabetes (He *et. al.,* 2011*)*. A study conducted in Thailand reported a significantly higher prevalence of metabolic syndrome in the tertile with the highest MSG intake. Furthermore, for every 1000 mg more MSG intake per day, the risk of having metabolic syndrome or being overweight increased, regardless of the individual's total energy intake or physical activity level (Insawang *et al.* 2012).

Epidemiological studies and animal studies demonstrate an association between MSG consumption/MSG-injected models and weight gain (Clough *et al.,* 1986; He *et al.,* 2008; Hirata *et al.,* 1997; Insawang *et al.,* 2012; Iwase *et al.,* 1998; Olney, 1969; Svidnicki *et al.,* 2013). Miskowiak *et al.* (1993) found that rats administered with MSG displayed abnormal growth, obesity and reduced mass of the pituitary glands and testes, and also lower testosterone levels.

Most known or suspected obesogens are endocrine disruptors (EDs). Many are widespread with exposure suspected or confirmed as quite common (Holtcamp, 2012). Metabolic disorders may be influenced by endocrine disrupting interactions between consumed MSG and the hormones involved in controlling satiety responses and insulin release. Glucagon-like peptide 1 (GLP-1) is an intestinal gut hormone with important physiological roles including appetite control and is key in the regulation of post-prandial increases in insulin secretion, thereby regulating levels of glycaemia (Baggio and Drucker, 2007; Drucker, 2006). GLP-1 mediates its effects through the GLP-1 receptor (GLP-1R) which is a G-protein coupled receptor (GPCR) expressed in a wide range of human tissues including α, β and δ- cells of the pancreatic islets, lung, kidney, heart, intestine, stomach, skin, vagal nerve and several regions of the central nervous system (CNS) including the hypothalamus and brainstem. GLP-1Rs

117 have been shown to rapidly internalise following activation by GLP. A study by Kuna et al.,
118 2013 demonstrated that GLP-1 receptor internalisation was induced by 0.1 μM GLP-1 2
119 minutes to 1 h. Internalised receptors are then either recycled back to the cell surface where
120 they can again engage with ligands, or are targeted for post-endocytic degradation, which
121 results in permanent signal termination from the receptor (Noerklit et al., 2014).

122 GLP-1 is well established to regulate bodyweight by stimulating feelings of satiety via GLP-
123 1R in the brain (Baggio and Drucker, 2007; Drucker, 2006). In addition to the most well
124 characterised effect, the amplification of glucose-stimulated insulin secretion, GLP-1 is now
125 known to induce expansion of insulin-secreting β-cell mass. The mechanism by which GLP-1
126 is believed to enhance insulin secretion is through the regulation of ion channels (including
127 ATP-sensitive K^+ channels, voltage-dependent Ca^{2+} channels, voltage-dependent K^+
128 channels, and nonselective cation channels) and by the regulation of intracellular energy
129 homeostasis and exocytosis (Macdonald et al., 2002). A number of clinical trials have been
130 conducted in order to investigate the weight loss effect of GLP-1R agonist therapies in
131 diabetic patients. A correlation between these therapies and weight loss has been established
132 with the GLP-1R agonist liraglutide having a significant positive effect on weight loss (Buse
133 *et al.,* 2013).

134 Hosaka *et al.,* (2012) investigated the effect of a lipid-containing meal with added MSG
135 (2000 mg/meal) on glucose homeostasis, incretin secretion and gastric emptying in humans.
136 GLP-1 plasma levels were significantly increased (although the mechanism for this was not
137 determined). One possible mechanism could be through the interaction of the GLP-1R. It is
138 well-established that sustained GLP-1R antagonism (with exendin (9-39)) impairs glucose
139 tolerance, decreases insulin secretion and causes hyperglycaemia (D'Alessio *et al.,* 1996;
140 Edwards *et al.,* 1999; Kolligs, Fehmann *et al.,* 2002; Green *et al.,* 2005; Scrocchi *et al.,*
141 1996). As MSG is proposed to be an obesogen it seems logical to investigate whether it can
142 disrupt the GLP-1/GLP-1R axis.

143 This is the first study assessing a potential obesogenic mechanism for MSG and the aims are
144 to primarily investigate 3 h and 72 h exposure effects of MSG on GLP-1 secretion *in vitro*
145 and also to investigate any potential mechanisms through 1) examining 3 h and 72 h toxicity
146 of MSG on enteroendocrine (EE) cells (pGIP/neo: STC-1 cells, a sub-clone of the STC-1 cell
147 line which secretes GLP-1) including measures of subtle pre-lethal cytotoxicity and 2)

148 studying the endocrine disrupting potential of MSG on the GLP-1 receptor using a GLP-1R
149 redistribution assay and high content analysis (HCA).

150

151 ## 2. Materials and methods

152 ### *2.1. Chemicals and reagents*

153 Cell culture reagents and Hank's Balanced Salt Solution (HBSS) were supplied by Life
154 Technologies (Paisley, UK). The reference standard GLP-1 (7-37) was obtained from Abcam
155 (Cambridge, UK). MSG, thiazolyl blue tetrazolium bromide (MTT) and formalin were all
156 supplied by Sigma-Aldrich (Poole, Dorset, UK). Hoechst nuclear stain and mitochondrial
157 membrane potential dye were provided by Thermo Scientific (UK). GLP-1 ELISA kits were
158 purchased from Millipore (Billerica, MA, USA). All other reagents were standard laboratory
159 grade.

160

161 ### *2.2. Cell culture*

162 The U2OS-GLP1R-EGFP cell line (a U2OS cell line with stable expression of the human
163 GLP-1R with a C-terminal EGFP tag) was obtained from Thermo Scientific (UK) and grown
164 in 75 cm^2 tissue culture flasks (Nunc, Roskilde, Denmark) at 37 °C with 5% CO_2 and 95%
165 humidity. The cells were cultured routinely in Dulbecco's modified Eagle medium (DMEM)
166 media with Glutamax, 10% foetal bovine serum (FBS), 1% penicillin and streptomycin (50
167 U/ml) and 50 mg/ml G418. Cells were grown in plating media (DMEM with Glutamax, 1%
168 FBS, 1% penicillin and streptomycin (50 U/ml)) for 24 h prior to running the assay. Test
169 compounds and standards were diluted in assay media (DMEM with Glutamax, 1% penicillin
170 and streptomycin (50 U/ml)). The assay has been validated with cells up to a passage of 30
171 (Thermo Scientific, UK).

172 pGIP/neo: STC-1 cells are an enteroendocrine (EE) cell model and its GLP-1 secretory
173 ability has been extensively investigated (Gillespie *et al.,* 2015; Jafri *et al.,* 2016). These were
174 a gift from Dr. B. Wice (Washington University of St. Louis) with permission from Dr. D.
175 Hanahan (University of California, San Francisco, CA). Cells were cultured DMEM with
176 Glutamax, 10% FBS, 1% penicillin and streptomycin (50 U/ml) and 50 mg/ml G418. Cells
177 were passaged at 80-90% confluence and used between passages 15-50.

178

179 *2.3. Cell viability*

180 In addition to visual inspection of cells under the microscope to evaluate cell morphology and
181 attachment, two cell viability assays were used to assess cytotoxic effects of MSG exposure.

182 *2.3.1. MTT*

183 The thiazolyl blue tetrazolium bromide (MTT) assay was used to monitor the cytotoxic
184 effects of MSG test concentrations in the U2OS-GLP1R-EGFP cell line. Viable cells convert
185 the soluble yellow MTT into insoluble purple formazan by the action of mitochondrial
186 succinate dehydrogenase. For the U2OS-GLP1R-EGFP cells, clear flat-bottomed 96-well
187 plates (Nunc, Roskilde, Denmark) were seeded with 6 x 10^4 cells and the test compound was
188 added after 24 h and incubated for a further 1 h. The supernatant was discarded and 50 µl of
189 MTT solution/well (5mg/ml stock in phosphate buffered saline (PBS) diluted in 1:2.5 in
190 assay media) was added and cells were incubated for a further 3 h. Again, supernatant was
191 removed and 200 µl of DMSO was added to each well (to dissolve the formazan crystals) and
192 incubated for a further 10 min with agitation at 37 °C. Optical density was measured using a
193 Sunrise spectrophotometer at 570 nm with a reference filter at 630 nm (TECAN,
194 Switzerland). Samples were tested in triplicate and in three independent exposures. Viability
195 was calculated as a percentage absorbance of the sample when compared with the absorbance
196 of the solvent control.

197

198 *2.3.2. Alamar Blue*

199 The viability of the pGIP/neo: STC-1 cells in the acute and chronic study was determined
200 using the AlamarBlue®. After removal of media for hormone analysis, 1 ml of 1:10 (v:v)
201 AlamarBlue® in cell culture medium was added to each well and incubated for 24 h. A 100
202 µl volume was then removed from each well and added into clear flat-bottomed 96-well
203 microtiter plates (BD Biosciences, Bedford, MA, US). Using a Sunrise spectrophotometer
204 (TECAN, Switzerland) the absorbance was measured at 570 nm and 600 nm. Viability was
205 calculated as the percentage absorbance of the sample in comparison with the absorbance of
206 the solvent control (0.1%, v:v dH_20 (deionised water) in media).

207

208 *2.4. GLP-1R redistribution assay*

209 U2OS-GLP1R-EGFP cells were seeded (100 µl of 6 x 10^4 cells/well) in 96-well black plates
210 with clear, flat bottoms. The cells were allowed to attach for 24 h before adding 100 µl of the
211 test sample (MSG) and the GLP-1 (7-37) standards at a final dH_2O and dimethyl sulfoxide
212 (DMSO) concentration of 0.2%. To assess whether MSG had a disruptive (or antagonistic)
213 effect on the interaction of GLP-1 with the GLP-1R, the potential of MSG to influence GLP-
214 1-mediated internalisation of the EGFP-tagged GLP-1R was assessed. Thus, the ability of
215 150 nM GLP-1 to induce internalisation of the receptor was assessed in the absence and
216 presence of MSG. Cells were incubated for 1 h in a 37 °C, 5% CO_2, humidified incubator,
217 before decanting buffer and fixation of the cells with 100 µl/well of formalin (approximately
218 4% formaldehyde). Cells were incubated for a further 20 min at room temperature and then
219 washed four times with 200 µl PBS per well per wash followed by addition of 100 µl of 1
220 µM Hoechst staining solution (1 µl: 10 ml PBS) to each well. After 30 min, the plate was
221 imaged on a Thermo Scientific Arrayscan HCS. The filters were set for the Hoechst dye
222 (350/461 nm) and GFP/FITC (488/509 nm). The SpotDetectorV3 BioApplication was used
223 for carrying out the reading step of the GLP-1R redistribution assay using the
224 ObjectSpotAvgIntensity parameter and both data and corresponding images are generated
225 (see *Fig 1* for example of images). The assay was performed in triplicate for each
226 experimental point and repeated in three independent exposures. The response of the cell line
227 to the various compounds was measured and compared with the dH_20 solvent control.

228

229 *2.5. HCA cytotoxicity assay*

230 The Cellomics® HCS reagent series multiparameter cytotoxicity assay was performed
231 according to the manufacturer's instructions. pGIP/neo: STC-1 cells were seeded 6 x 10^4 in
232 96 well plates 24 hours prior to the assay. Briefly, mitochondrial membrane potential dye was
233 prepared by adding 117 µl of anhydrous DMSO to make a 1 mM stock. Following incubation
234 (3 h or 72 h), 50 µl of live cell stain was added to each well for 30 min at 37 °C and protected
235 from light. The live stain was removed and cells were then fixed with a 10% formalin
236 solution for 20 min at room temperature, protected from light and washed with PBS. Hoechst
237 33342 dye at a final concentration of 1.6 µM was added to each well and incubated for 10
238 min at room temperature and protected from light; after which cells were washed with PBS
239 four times and evaluated on a CellInsight^{TM} NXT High Content Screening (HCS) Platform

240 (Thermo Fisher Scientific, UK). Hoechst stain was used to measure cell number and nuclear
241 morphology including nuclear intensity and nuclear area while mitochondrial membrane
242 potential dye was used to measure mitochondrial health, specifically mitochondrial
243 membrane potential and mitochondrial mass. Data were captured for each plate using a $\times$ 10
244 objective magnification in the selected excitation and emission wavelengths of Hoechst dye
245 (Ex/Em 350/461 nm) and mitochondrial membrane potential dye (Ex/Em 554/576 nm). For
246 each well, 25 field of view images were acquired to examine each parameter with each image
247 containing approximately 6,000 cells.

248

249 *2.6. GLP-1 secretion studies*

250 *2.6.1. 3 h exposure*

251 The pGIP/neo: STC-1 cells were seeded into 24-well plates (1 x 10^6 per well) and cultured
252 overnight at 37 °C in a humidified atmosphere of 5% CO_2. Medium was removed and cells
253 were washed once with HBSS buffer and equilibrated for 1 h in 500 µl HBSS buffer. Test
254 compounds (0.5, 5, 50, 250, 500 µg/ml MSG in dH_20) and dH_20 solvent control diluted in
255 HBSS buffer (1000 µl) were added and allowed to incubate for a further 3 h. Supernatant was
256 removed and stored at -20°C prior to hormone analysis.

257

258 *2.6.2. 72 h exposure*

259 Initially cells were seeded into 96 well plates (5 x 10^4 per well) and cultured overnight at 37
260 °C in a humidified atmosphere of 5% CO_2. Test concentrations (0.5, 5, 50, 250, 500 µg/ml
261 MSG in dH_20) and dH_20 solvent control diluted in reduced serum media (1% serum) (100 µl)
262 were added and allowed to incubate for 72 h. Media from each well was harvested and
263 retained at 24 h intervals and replaced by fresh media containing the appropriate
264 concentration of MSG or vehicle control. Samples were stored at -20 °C until immediately
265 before assay of the GLP-1 content at which time the 3 x 200 µl samples from each well were
266 pooled.

267

268 *2.7. Hormone Analysis*

269 GLP-1 was measured using a Glucagon Like Peptide-1 (GLP-1) (Active) ELISA kit (EGLP-
270 35K; in accordance with the manufacturer's instructions (Millipore, Watford, UK). All
271 experiments were performed in triplicate for each experimental point and repeated in three
272 independent experiments. Responses of cells to the test compound were compared with the
273 negative control (dH_20, 0.1%).

274

275 *2.8. Statistical Analysis*

276 All values shown are expressed as mean ± standard error of the mean (SEM) of the three
277 independent exposures for MSG. Data from the cell viability, GLP-1R redistribution assay
278 and GLP-1 ELISA were analysed using Microsoft Excel and GraphPad PRISM 5 software
279 (GraphPad Software Inc, San Diego, CA). A one-way analysis of variance (ANOVA)
280 followed by Dunnett's multiple comparison test was used to determine significant differences
281 between treatments and the corresponding control. A P-value of < 0.05 was considered as
282 significant ($P < 0.05$ *, $P < 0.01$ ** and $P < 0.001$ ***).

283 **3. Results**

284 *3.1. Cell viability data*

285 Viability of the pGIP/neo: STC-1 cells was not affected by any concentration of MSG during
286 either the acute or the chronic study, as determined by AlamarBlue® assay ($P > 0.05$) (data
287 not shown). Although not significantly different, there was some evidence of reduced
288 viability at higher concentrations of MSG at 72 h. However as this is a relatively insensitive
289 assay there was sufficient justification for looking at potential cytotoxic markers on HCA.

290 Similiarly, MTT assays showed no significant change in viability of the U2OS-GLP1R-EGFP
291 cells following 1 h exposure to MSG (0.5, 5, 50, 250 and 500 μg/ml) (data not shown).

292

293 *3.2. Effects of MSG on GLP-1 secretion*

294 *3.2.1. Effects of 3 h exposure of MSG on GLP-1 secretion*

295 Following 3 h exposure of pGIP/neo: STC-1 cells to lower MSG concentrations (0.5-250
296 μg/ml) did not significantly affect GLP-1 secretion. However, at 500 μg/ml, MSG stimulated

a significant 9.6 pg /10^6 increase in GLP-1 secretion (22.4 pg /10^6 cells in 500 µg/ml MSG vs. 12.8 pg /10^6 cells in control; $P < 0.001$) (*Fig.2*).

3.2.2. Effects of 72 h exposure of MSG on GLP-1 secretion

Similarly 72 h exposure of pGIP/neo: STC-1 cells to lower MSG concentrations (0.5-250 µg/ml) did not significantly affect GLP-1 secretion. However, at 500 µg/ml MSG there was a significant decrease in GLP-1 secretion by 35.9 pg/10^6 cells (45.4 pg /10^6 cells in 500 µg/ml MSG vs 81.4 pg /10^6 cells in control $P < 0.05$) (*Fig.3*).

3.3. Cytotoxicity

The potential cytotoxicity of either 3 h or 72 h exposure to MSG (0.5, 5, 50, 250 and 500 µg/ml) on pGIP/neo: STC-1 intestinal cells was assessed by HCA analysis by measuring cell number and nuclear morphology including nuclear intensity, and nuclear area.

Mitochondrial membrane potential dye was used to measure mitochondrial health, specifically mitochondrial membrane potential and mitochondrial mass. For 3 h MSG exposure no cytotoxicity was observed at any MSG concentrations (data not shown).

However, following 72 h exposure, 250 µg/ml MSG decreased cell number by 11.8% ($P < 0.05$), increased nuclear area by 23.9% ($P < 0.001$) and decreased mitochondrial membrane potential by 13% ($P < 0.05$). MSG (500 µg/ml) decreased cell number by 26.7% ($P < 0.001$), increased nuclear area by 29.8% ($P < 0.001$), increased mitochondrial mass by 16.3% ($P < 0.01$) and decreased mitochondrial membrane potential by 21.6% ($P < 0.001$) (*Fig. 4 and 5*).

3.4. GLP-1R internalisation assay

A standard curve was generated using various concentrations of GLP-1 (1-300 nM) to drive receptor internalisation (*Fig.6*). The EC_{50} value was calculated as 37 nM GLP-1.

MSG did not cause internalization of the GLP-1R in U2OS-GLP1R-EGFP cells. Furthermore, exposure to MSG (0.5-500 µg/ml) during the period of exposure to GLP-1 did not affect GLP-1-mediated internalisation of the GLP-1R (Figure 7).

325

326 ## 4. Discussion

327 Associations between MSG consumption and increased incidence of diabetes and obesity
328 have been reported in both human and animal studies (He *et al.,* 2011; Insawang *et al.* 2012;
329 Miskowiak *et al.,* 1993). However, a potential mechanism has not yet been identified. This
330 study hypothesised that MSG might interact with the GLP-1 hormone, either through the
331 GLP-1R or by affecting GLP-1 secretion. For an obesogen, GLP-1 is a plausible potential
332 target given its pleiotropic actions on satiety responses, post-prandial insulin and glucagon
333 secretion, gut motility, and gastric acid secretion.The present study investigated whether
334 MSG has a disrupting effect on the secretion of the incretin hormone GLP-1 or its interaction
335 with its receptor. Cytotoxic responses were evaluated in pGIP/neo: STC-1 cells, an EE cell
336 model which secretes GLP-1. EE cells are one of four subtypes of epithelial cells lining the
337 gut (Beucher *et al.,* 2012) and collectively are the largest endocrine system in the body. The
338 cells used in this study (pGIP/neo: STC-1) are a sub-clone of the STC-1 cell line which
339 secretes GLP-1 (Gillespie *et al.,* 2015; Jafri *et al.,* 2016). Recent studies suggest that EE cells
340 are more flexible than previously thought because they alter the profile of hormones made
341 and released in response to nutrient changes in the diet (Habib *et al.* 2012). As pGIP/neo:
342 STC-1 cells are a pluri-hormonal EE cell line (also secreting cholecystokinin, peptide YY and
343 glucose-dependent insulinotropic polypeptide) (Hand *et al.,* 2012) that imitate some of the
344 other hormonal influences which exist in the intestine *in vivo* such as having the potential to
345 release other gut hormones.

346 A previous study on the STC-1 parental cells provides evidence that exposure to MSG can
347 activate the nutrient sensing T1R1/T1R3 (taste receptors) umami receptor. Additionally,
348 MSG exposure to the STC-1 cells induced neurotensin (NT) release, a peptide involved in the
349 regulation of hormone release from the gut (Kendig et al., 2015). However, in the present
350 study the potential of MSG to interfere with GLP-1 interaction with the GLP-1R was
351 examined and under all of the various test conditions there was no evidence of this. It is
352 important to mention that the cell lines used for the present study and the Kendig et al.,
353 (2015) study differ,as well as the receptor investigated, therefore accounting for differences
354 in findings.

355 When studying the effects of MSG on intestinal EE cells, which produce and release GLP-1,
356 3 h exposure of MSG stimulated GLP-1 secretion with a concentration 500 μg/ml almost

357 doubling the amount of hormone that is released. Previously, it has been demonstrated *in vivo*
358 that an acute bolus of MSG increases plasma GLP-1 in healthy male volunteers (Hosaka *et al.*
359 2012). The present data suggest that this could be a consequence of a direct effect of MSG on
360 GLP-1 secretion by EE cells.

361 Secretion of GLP-1 occurs primarily in response to nutrient ingestion, particularly glucose
362 and fat and to a lesser extent specific amino acids and protein hydrosylates (Elliott *et al.,*
363 1993, Ramshur *et al.,* 2002). However, it may not solely be the presence of nutrients that
364 stimulates secretion. A biphasic release of GLP-1 into the circulation following a meal has
365 been established (Rask *et al.,* 2001) which would suggest the potential input of neural and
366 endocrine factors. It is believed that GLP-1 secretion may prove even more complex than
367 other gut hormones. In particular, glutamine, a breakdown product of MSG, may be even
368 more effective than glucose or other amino acids as a GLP-1 secretagogue. Glutamine
369 triggers membrane depolarisation and increases intracellular calcium; however asparginine
370 and alanine also increase intracellular calcium without increasing GLP-1 secretion (Reimann
371 *et al.,* 2004). In the presence of diazoxide (a potassium channel activator) and a depolarising
372 concentration of potassium chloride, glutamine still enhanced GLP-1 secretion (Reimann *et*
373 *al.,* 2004), therefore glutamine-induced GLP-1 secretion may not be exclusively dependent
374 on membrane polarisation. Therefore, the mechanism by which MSG induced an increase in
375 GLP-1 secretion following 3 h exposure, may have been consequential of the breakdown
376 product of MSG, glutamine. The paracrine actions of somatostatin, secreted from
377 neighbouring D-cells exerts an inhibiting effect on GLP-1 secretion and may prove pivotal in
378 producing a feedback loop for GLP-1 regulation following nutrient ingestion (Hansen *et al.,*
379 2000; Lahlou *et al.,* 2004). Previous studies using the parental STC-1 cell line have
380 demonstrated that exposure to glutamine elevated the intracellular Ca^{2+} levels (Miyata et al.,
381 2014). It may be via this mechanism that the GLP-1 secretion is increased following 3 hour
382 exposure to higher concentrations of MSG

383 MSG is consumed on a daily basis and is present in a wide variety of foods and therefore it
384 was logical to examine the effects of a longer exposure on GLP-1 secretion (72 h) mimicking
385 daily consumption. When pGIP/neo STC-1 cells were exposed to MSG for 72 h ,a
386 detrimental effect on GLP-1 secretion leading to a 1.8-fold reduction in the amount of
387 hormone released was observed. A possible explanation for the chronic decrease in GLP-1
388 secretion is that MSG-induced stimulation of GLP-1 secretion (as observed with 3 h
389 exposure) leads to a depletion in the capacity of EE cells to secrete GLP-1. However this

390 would need to be studied in more detail with further investigations. This observation has
391 potential physiological relevance given that GLP-1 secretion may be blunted/impaired in
392 patients with type 2 diabetes. For example, reduced postprandial concentrations of intact
393 biologically active GLP-1 have been observed in type 2 diabetic patients and it seem likely
394 that that these reductions in GLP-1 secretion result in impaired insulin secretion (Vilsbøll *et*
395 *al.,* 2001). Therefore our observation that 72 h MSG exposure leads to a decline in GLP-1
396 secretion suggests that it may be a contributing factor in the pathogenesis of diabetes/obesity.

397 A novel HCA assay measuring nuclear and mitochondrial parameters was developed to
398 assess the cellular health of EE cells under conditions of both 3 h and 72 h MSG exposure.
399 No cytotoxicity was evident when the EE cells were exposed to MSG for 3 h, however,
400 significant toxic effects were observed following 72 h exposure. Concentration-dependent
401 reductions in cell number and in mitochondrial membrane potential were observed, as were
402 increases in nuclear area and mitochondrial mass.

403 MSG exposure studies are scarce in the literature. However, several *in vivo* single-dose
404 studies have shown the potential of MSG to impact negatively upon cells and ultimately lead
405 to cell death. One study investigated the exposure of rat thymocytes to MSG (4 mg/g body
406 weight) and found a dose-dependent decrease in cell survival with cell loss occurring
407 primarily through apoptosis (Pavlovic et. al., 2009). Increased apoptosis can arise from the
408 absence of survival factors and cell-to-cell signalling mechanisms that are present *in vivo*
409 (Elmore, 2007). The concentration-dependent manner in which MSG induces apoptosis has
410 been noted previously whereby glutamate-induces cell death via apoptosis or necrosis
411 (Ankarcrona et. al., 1995). The results of the present study provide no evidence of 3 h toxic
412 effects, but again this can be due to a number of other factors including the cell type used, the
413 MSG concentrations employed and the duration of exposure.

414 In the present study there was a good association between the MSG concentrations that
415 caused decline in GLP-1 secretion and those which caused cytotoxic effects. At the highest
416 MSG concentration tested (500 µg/ml) there was a significant decrease in both cell number
417 and in mitochondrial membrane potential but a significant increase in mitochondrial mass and
418 nuclear area. It is well established that biogenesis of mitochondria can increase mitochondrial
419 mass as a result of increased mitochondrial respiration, and that this usually corresponds with
420 reduced mitochondrial membrane potential (O'Brien and Haskins, 2007). Mitochondria are
421 organelles involved in the regulation of programmed cell death (apoptosis). This often occurs

422 when cells are damaged by disease or toxic agents (Norbury and Hickson, 2001). Due to the
423 increase in nuclear area observed in the study, it would appear that some cells underwent
424 necrosis. In the case of necrosis, the cells will swell and consequently cause an increase in
425 nuclear area, as observed in the present study. Although there are distinct differences in the
426 mechanisms of apoptosis and necrosis, there is overlap between the two processes described
427 as the "apoptosis-necrosis continuum" (Zeiss, 2003) and the results of the HCA assays
428 suggest that the highest concentration (500 μg/ml) of MSG is capable of inducing "apoptosis-
429 necrosis continuum" in EE cells during the 72 h exposure.

430 The concentrations of MSG used in this study were based on dietary exposure levels, average
431 daily intakes across different populations and at levels higher than these levels to account for
432 underestimations in MSG intake (Collision *et al.,* 2009; He *et al.,* 2011; Nakanishi *et al.,*
433 2008; Shi *et al.,* 2014). Maximum MSG solubility was achieved at 500 mg/ml, giving a top
434 concentration on the assay plate of 500 μg/ml, equivalent to an exposure of 30 g/day.
435 Although this is much higher than average daily intakes discussed, as outlined in the
436 introduction, restaurants add unknown amounts of MSG to foods, therefore it is difficult to
437 estimate accurate exposure and it is therefore possible certain groups of the population could
438 be exposed to these high levels, where the present study found significant effects.
439 Furthermore, in a society where MSG is not regulated and consumers are increasingly eating
440 out or purchasing take-away foods, it is not unreasonable to suggest that MSG exposure is
441 likely to be on the increase. It is also worth mentioning the potentially greater health risks
442 which Asian populations are being subjected to as a result of their excessive MSG exposure.
443 Large scale epidemiological studies are clearly warranted to provide better estimates of MSG
444 consumption. The setting of maximum levels of MSG content in foods is something which
445 should be given serious consideration. This would have the added benefit of improving the
446 accuracy of consumer exposure for this food additive.

447

448 **Conclusion**

449 This is the first study to examine a potential endocrine disrupting effect of MSG, a suspected
450 obesogen, on the interaction with the GLP-1 hormone. This study demonstrates that MSG,
451 has the ability to stimulate GLP-1 secretion from pGIP/neo: STC-1 cells, an EE cell model
452 following 3 h exposure, but that longer-term exposure leads to impaired GLP-1 secretion and
453 a range of changes indicative of cytotoxicity. The multi-parameter HCA cytotoxicity assay

utilised here was able to detect subtle changes in cell health and this has great potential for investigating the potential endocrine disrupting effects of other putative obesogens.

Conflict of interest

There is no conflict of interest.

References

Ankarcrona, M., Dypbukt, J. M., Bonfoco, E., Zhivotovsky, B., Orrenius, S., and Lipton, S. A. (1995). Glutamate-Induced Neuronal Death: A Succession of Necrosis or Apoptosis Depending on Mitochondrial Function. *Neuron*, *15*, 961–973

Baggio, L. and Drucker, D. (2007). Biology of Incretins: GLP-1 and GIP. *Gastroenterology*, *132*, 2131–2157.

COT(2013). Scoping paper on the obesogen hypothesis (pp. 1–23).Available from: https://cot.food.gov.uk/sites/default/files/cot/tox2013.pdf (Accessed: 12/06/16).

Beucher, E. Gjernes, C. Collin, M. Courtney, A. Meunier, and P. Collombat, (2012).The homeodomain-containing transcription factors Arx and Pax4 control enteroendocrine subtype specification in mice. *PLoS One*, 7 (5) e36449

Buse J., Nauck M., Forst T., Sheu W., Shenouda S., Heilmann C., et. al., (2013) Exenatide once weekly*versus* liraglutide once daily in patients with type 2 diabetes (DURATION-6): a randomised, open-label study. Lancet 381: 117–124

Clough, R., Ararich, P., and Sladek, C.(1986). Monosodium glutamate neurotoxicity: A sex-specific impairment of blood pressure but not vasopressin in developing rats. *Brain Research Bulletin*, *17*(1), 51–58.

Collison, K. S., Maqbool, Z., Saleh, S. M., Inglis, A., Makhoul, N. J., Bakheet, R., and Al-Mohanna, F. A. (2009). Effect of dietary monosodium glutamate on trans fat-induced nonalcoholic fatty liver disease. *Journal of Lipid Research*, *50*, 1521–1537.

D'Alessio, D. a, Vogel, R., Prigeon, R., Laschansky, E., Koerker, D., Eng, J., and Ensinck, J. W. (1996). Elimination of the action of glucagon-like peptide 1 causes an impairment of

480 glucose tolerance after nutrient ingestion by healthy baboons. *The Journal of Clinical*
481 *Investigation*, *97*(1), 133–8.

482 Drucker, D. J. (2006). The biology of incretin hormones. *Cell Metabolism*, *3*(3), 153–65.

483 Edwards, C., Todd, J.and Mahmoudi, M. (1999). Glucagon-like peptide 1 has a physiological
484 role in the control of postprandial glucose in humans: studies with the antagonist exendin 9-
485 39. *Diabetes*, *48*, 86–93.

486 Elliott, R. M., Morgan, L. M., Tredger, J. A., Deacon, S., Wright, J., and Marks, V. (1993).
487 Glucagon-like peptide-1(7-36)amide and glucose-dependent insulinotropic polypeptide
488 secretion in response to nutrient ingestion in man - acute postprandial and 24-H secretion
489 patterns. Journal of Endocrinology, 138, 159-166.

490 Elmore, S. (2007). Apoptosis: a review of programmed cell death. *Toxocologic Pathology*,
491 *35*(4), 495–516

492 FDA. (2012). Questions and Answers on Monosodium glutamate (MSG). Retrieved January
493 06, 2015, from
494 http://www.fda.gov/Food/IngredientsPackagingLabeling/FoodAdditivesIngredients/ucm3287
495 28.htm

496 Gillespie, A. L., Calderwood, D., Hobson, L., and Green, B. D. (2015). Whey proteins have
497 beneficial effects on intestinal enteroendocrine cells stimulating cell growth and increasing
498 the production and secretion of incretin hormones. *Food Chemistry*, *189*, 120–128

499 Green, B. D., Irwin, N., Gault, V. a., Bailey, C. J., O'Harte, F. P. M., and Flatt, P. R. (2005).
500 Chronic treatment with exendin(9-39)amide indicates a minor role for endogenous glucagon-
501 like peptide-1 in metabolic abnormalities of obesity-related diabetes in ob/ob mice. *Journal*
502 *of Endocrinology*, *185*, 307–317

503 Habib, AM, Richards, P, Cairns, LS, Rodger, GJ, Bannon, CA and Parker, HE. (2012).
504 Overlap of endocrine hormone expression in the mouse intestine revealed by transcriptional
505 profiling and flow cytometry. *Endocrinology.* 153 (7), p3054-3065.Hand, K. V, Giblin, L.,
506 and Green, B. D. (2012). Hormone profiling in a novel enteroendocrine cell line pGIP/neo:
507 STC-1. *Metabolism: Clinical and Experimental*, *61*(12), 1683–6.

508 Hand, K. V, Giblin, L., and Green, B. D. (2012). Hormone profiling in a novel
509 enteroendocrine cell line pGIP/neo: STC-1. *Metabolism: Clinical and Experimental*, *61*(12),
510 1683–6.

511 Hansen, L., Hartmann, B., Bisgaard, T., Mineo, H., Jorgensen, P. N., and Holst, J. J. (2000).
512 Somatostatin restrains the secretion of glucagon-like peptide-1 and-2 from isolated perfused
513 porcine ileum. American Journal of Physiology-Endocrinology and Metabolism, 278, E1010-
514 E1018

515 He K, Zhao L, Daviglus ML, Dyer AR, Van Horn L, Garside D, Zhu L, Guo D, Wu Y, Zhou
516 B, Stamler J (2008) Association of monosodium glutamate intake with overweight in Chinese
517 adults: the INTERMAP study. *Obesity* 16:1875–1880

518 He, K., Du, S., Xun, P., Sharma, S., Wang, H., Zhai, F., and Popkin, B. (2011). Consumption
519 of monosodium glutamate in relation to incidence of overweight in Chinese adults : China
520 Health and Nutrition Survey (CHNS) *American Journal of Clinical Nutrition*, 1 – 3, 1–3.

521 Hirata, E., Andrade, I. S., Vaskevicius, P., and Dolnikoff, M. S. (1997). Monosodium
522 glutamate (MSG)-obese rats develop glucose intolerance and insulin resistance to peripheral
523 glucose uptake. *Brazilian Journal of Medical and Biological Research*, *30*(5), 671–4.

524 Holtcamp, W. (2012). Obesogens: An environmental link to obesity. *Environmental Health*
525 *Perspectives*, *120*(2), a62–a68.

526 Hosaka, H, Kusano, M and Zai, H. (2012). Monosodium glutamate stimulates secretion of
527 glucagon-like peptide-1 and reduces postprandial glucose after a lipid-containing meal.
528 *Alimentary pharmacology and Therapeutics.* 36 (1), p95-903.

529 Husarova, V., and Ostatnikova, D. (2013). Monosodium Glutamate Toxic Effects and Their
530 Implications for Human Intake: A Review. *JMED Research*, *2013*, 1–12.

531 Insawang, T., Selmi, C., Cha'on, U., Pethlert, S., Yongvanit, P., Areejitranusorn, P., and
532 Hammock, B. D. (2012). Monosodium glutamate (MSG) intake is associated with the
533 prevalence of metabolic syndrome in a rural Thai population. *Nutrition & Metabolism*, *9*(1),
534 50.

535 Iwase, M., Yamamoto, M., Iino, K., Ichikawa, K., Shinohara, N., Yoshinari, M., and
536 Fujishima, M. (1998). Obesity induced by neonatal monosodium glutamate treatment in
537 spontaneously hypertensive rats: an animal model of multiple risk factors. *Hypertension*
538 *Research : Official Journal of the Japanese Society of Hypertension*, *21*(1), 1–6.

539 Jafri, L., Saleem, S., Calderwood, D., Gillespie, A., Mirza, B., and Green, B. D. (2016).
540 Naturally-occurring TGR5 agonists modulating glucagon-like peptide-1 biosynthesis and
541 secretion. Peptides, 78, 51–58.

542 Kendig, D., Bala, V., Murthy, K. and Grider, J., (2015) Neurotensin Partially Mediates the
543 Monosodium Glutamate (MSG)-Induced Peristaltic Reflex. *The FASEB Journal,* 29(1
544 Supplement), pp.850-1.

545 Kolligs, F., Fehmann, H., Goke, R., and Goke, B. (2002). Reduction of the incretin effect in
546 rats by the glucagon-like peptide 1 receptor antagonist exendin (9-39) amide. *Diabetes*, *44*,
547 16–19.

548 Kuna, R. S., Girada, S. B., Asalla, S., Vallentyne, J., Maddika, S., Patterson, J. T and Mitra,
549 P. (2013). Glucagon-like peptide-1 receptor-mediated endosomal cAMP generation promotes
550 glucose-stimulated insulin secretion in pancreatic NL -cells. *American Journal of Physiology -*
551 *Endocrinology and Metabolism,* 305 (2), E161–E170.

552 Lahlou, H., Guillermet, J., Hortala, M., Vernejoul, F., Pyronnet, S., Bousquet, C., and Susini,
553 C. (2004). Molecular signaling of somatostatin receptors. Gastroenteropancreatic
554 Neuroendocrine Tumor Disease: Molecular and Cell Biological Aspects, 1014, 121-131.
555 *creatic Neuroendocrine Tumor Disease: Molecular and Cell Biological Aspects, 1014*, 121-
556 131.

557 Macdonald, P. E., El-kholy, W., Riedel, M. J., Salapatek, A. M. F., Light, P. E., and Wheeler,
558 M. B. (2002). The Multiple Actions of GLP-1 on the Process of Glucose-Stimulated Insulin
559 Secretion, Diabetes *51* S434-S442

560

Mercola (2009) MSG: Is This Silent Killer Lurking in Your Kitchen Cabinets. Available from: http://articles.mercola.com/sites/articles/archive/2009/04/21/msg-is-this-silent-killer-lurking-in-your-kitchen-cabinets.aspx. Accessed: 12/06/16

Miskowiak B., Limanowski A., and Partyka M. (1993): Effect of perinatal administration of monosodium glutamate (MSG) on the reproductive system of the male rat. *Endokrynologia Polska 44*, 497–505

Miyata, M., Kurogi, M., Oda, M. and Saitoh, O., (2014) Effect of five taste ligands on the release of CCK from an enteroendocrine cell line, STC-1. *Biomedical Research*, 35(2), pp.171-176.Newbold, R. R., Padilla-banks, E., Snyder, R. J., Phillips, T. M., and Jefferson, W. N. (2007). Developmental exposure to endocrine disruptors and the obesity epidemic. *National Institute of Health*, *23*(3), 290–296.

Kuna, R. S., Girada, S. B., Asalla, S., Vallentyne, J., Maddika, S., Patterson, J. T., and Mitra, P. (2013). Glucagon-like peptide-1 receptor-mediated endosomal cAMP generation promotes glucose-stimulated insulin secretion in pancreatic β -cells. *American Journal of Physiology - Endocrinology and Metabolism,* 305(2), E161–E170.

Noerklit, S., Wismann, P., Rye, C., Kulahin, N., Iversen, H., Arevad, K.,and Waldhoer, M. (2014). Molecular and Cellular Endocrinology Real-time trafficking and signaling of the glucagon-like peptide-1 receptor. *Molecular and Cellular Endocrinology,* 382 (2), 938–949.

Norbury, C.J., and Hickson, I.D., (2001). Cellular responses to DNA damage. *Annual Reviews in Pharmacology and Toxicology*. *41*, 367–401

O'Brien, P., (2008). High content analysis of sub lethal cytotoxicity in human HepG2 hepatocytes for assessing potential and mechanism for chemical and drug induced human toxicity. High Content Screening (Science, Techniques, and Applications) (Haney, S.A). Wiley, NJ, pp. 293–315

587 O'Brien, P., and Haskins, J.R., (2007). In vitro cytotoxicity assessment. In: Taylor, D.L.,
588 Haskins, J.R., Giuliano, K.A. (Eds.), High Content Screening (Methods in Molecular
589 Biology). Humana Press, NJ, USA, pp. 415–425.

590 Olney, J. (1969). Brain lesions, obesity, and other disturbances in mice treated with
591 monosodium glutamate. *Science.*, *164*(880), 719–721.

592 Pavlovic, V, Pavlovic, D and Kocic, A. (2009). Ascorbic acid modulates monosodium
593 glutamate induced cytotoxicity in rat thymus. *Bratislavske Lekarske Listy.* 110 (4), p205-209.

594 Ramshur, E. B., Rull, T. R., and Wice, B. M. (2002). Novel insulin/GIP co-producing cell
595 lines provide unexpected insights into gut K-cell function in vivo. Journal of Cellular
596 Physiology, 192, 339-350.

597 Rask, E., Olsson, T., Soderberg, S., Johnson, O., Seckl, J., Holst, J. J., and Ahren, B. (2001).
598 Impaired incretin response after a mixed meal is associated with insulin resistance in
599 nondiabetic men. Diabetes Care, 24, 1640-1645.

600 Reimann, F., Williams, L., Xavier, G. D., Rutter, G. A., and Gribble, F. M. (2004).
601 Glutamine potently stimulates glucagon-like peptide-1 secretion from GLUTag cells.
602 Diabetologia, 47, 1592-1601.

603 Scrocchi, L., Brown, T., and MacLusky, N. (1996). Glucose intolerance but normal satiety in
604 mice with a null mutation in the glucagon-like peptide receptor gene. *Nature Medicine*, *2*,
605 1254–1258.

606 Sharpe, R. M., and Drake, A. J. (2013). Obesogens and obesity--an alternative view? *Obesity*
607 *(Silver Spring, Md.)*, *21*(6), 1081–3.

608 Shaw, J. E., Sicree, R. a., and Zimmet, P. Z. (2010). Global estimates of the prevalence of
609 diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice*, *87*, 4–14.

610 Svidnicki PV, de Carvalho Leite N, Venturelli AC, Camargo RL, Vicari MR, de Almeida
611 MC, Artoni RF, Nogaroto V, Grassiolli S. (2013) Swim training restores glucagon-like
612 peptide-1 insulinotropic action in pancreatic islets from monosodium glutamate-obese rats.
613 *Acta Physiologica* 209(1):34-44.

Vilsbøll, T., Krarup, T., Deacon, C. F., Madsbad, S., & Holst, J. J. (2001). Reduced Postprandial Concentrations of Intact Biologically Active Glucagon-Like Peptide 1 in Type 2 Diabetic Patients. *Diabetes*, *50*, 609–613.

Zeiss, C.J., 2003. The apoptosis-necrosis continuum: insights from genetically altered mice. *Veterinary Pathology 40* (5), 481–495.

Figure legends

Fig. 1: Images illustrating the GLP-1R redistribution. A) DMSO control and B) treated with agonist GLP-1 (300 nM). Typical HCA micrographs are shown with nuclei (blue) and GLP-1R fluorescence (green) X 20 objective magnification. 1B shows GLP-1R-EGFP internalisation that is detected by the image analysis algorithm.

Fig. 2. GLP-1 secretory responses of pGIP/neo: STC-1 cells during acute (3 h) exposure to MSG. Graph shows GLP-1 secretion from pGIP/neo: STC-1 cells (mean ± SEM, n = 3) following 3 h incubation with 0.5-500 µg/ml MSG.

Fig. 3. GLP-1 secretory responses of pGIP/neo: STC-1 cells *during chronic (72 h) exposure to MSG. Graph shows GLP-1 secretion from pGIP/neo: STC-1 cells (mean ± SEM, n = 3) following 72 h incubation with 0.5-500 µg/ml MSG. $P < 0.05$ (*) represent significance.*

Fig. 4: Cytotoxic effects on pGIP/neo STC-1 cells of MSG 0.5- 500 µg/ml following 72 h exposure. A number of endpoints were measured including a) Cell number, b) Nuclear area, c) Nuclear Intensity, d) Mitochondrial mass and e) Mitochondrial membrane potential. Data are expressed as a percentage of solvent control (dH_20) for each parameter. Data are mean ± SEM, n=3; $P < 0.05$ (*) < 0.01 (**) < 0.001 (***) versus appropriate control.

Fig. 5: HCA images for a) solvent control and b) MSG-treated (500 µg/ml) following 72 h exposure of pGIP/neo: STC-1 cells. Each image was acquired at × 10 objective magnification using Hoechst dye (blue; nuclear staining) and mitochondrial potential dye (red; mitochondrial staining).

Fig. 6: GLP-1 standard curve (1-300 nM) relative to the DMSO negative control in the U2OS-GLP1R-ERFP cell line. Data are mean±sem, n=3. GLP-1R internalisation was normalised to vehicle control (0.2% DMSO) and 300 nM GLP-1 (100%). The EC_{50} value was calculated at 37 nM.

643 *Figure 7: Images illustrating the GLP-1R redistribution assay following MSG exposure. A)*
644 *DMSO control, B) treated with MSG 500 µg/ml C) Positive control (GLP-1 150 nM) and D)*
645 *MSG 500 µg/ml and GLP-1 150 nM. Typical HCA micrographs are shown with nuclei (blue)*
646 *and GLP-1R fluorescence (green) X 20 objective magnification.*