The impact of food ingestion and acute administration of the GLP-1 receptor agonist exenatide on the brain's responses to food cues in type 2 diabetes

Short running title: Exenatide and brain responses to food in diabetes

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Abbreviations: BOLD blood oxygenation level dependent; DLPFC dorsolateral prefrontal cortex, fMRI functional magnetic resonance imaging; GLP-1 glucagon-like peptide 1.

Abstract

Differences in central responses to eating may underlie inter-individual differences in risk for obesity and type 2 diabetes. We investigated the impact of feeding and of the GLP-1 receptor agonist, exenatide, on regional brain responses to food cues. In a 2x2 factorial designed, randomly ordered, crossover study, 12 people with type 2 diabetes underwent Blood Oxygenation Level Dependent magnetic resonance neuroimaging whilst viewing images of food and non-food objects after ingestion of a 554kcal mixed meal (FED) or 50ml water (FASTED), and double-blind subcutaneous injection of 10mcg exenatide or placebo. Twelve non-diabetic subjects also undertook FED and FASTING neuroimaging, but without study medication. Diabetic subjects showed no change in hunger or fullness scores after eating. Exenatide was associated with reduced desire to eat at baseline; increased reward pathway activation by food cues during FASTING and decreased activation in higher executive brain regions after feeding. Non-diabetic subjects showed changes in fullness and hunger after food and activation of dorsolateral prefrontal cortex and insula on food viewing in FASTED only. Exenatide alters brain responses to food cues in type 2 diabetes in ways likely to enhance executive inhibition, which may contribute to its ability to reduce weight gain and improve diabetes control.

The current pandemic of type 2 diabetes is driven by rising rates of obesity, attributed to consumption of readily available, appetising, high-energy foods and declining need for exercise (1). Because of the strong association between obesity and type 2 diabetes, modulating food intake is a legitimate therapeutic target. An hypothesised dysregulation of appetite control which included failure of satiation and/or satiety (the desire to stop eating and/or the lack of desire to eat again) would increase risk of weight gain and worsening metabolic control in people with type 2 diabetes (2). Knowledge of the pathways involved in generating appetite control remains incomplete. Known satiety signals include a number of gut peptides, released in response to food ingestion, and also present in the brain, including glucagon-like peptide 1 (GLP-1) (3). GLP-1, is an incretin hormone secreted by L-cells in the distal small intestine, which contributes to the enhanced insulin response to glucose administered orally rather than intravenously (4). In people with type 2 diabetes, in whom GLP-1 responses to food are impaired (5), administration of GLP-1 receptor agonists enhances insulin responses to food, normalises food-induced suppression of glucagon, delays gastric emptying, improves insulin sensitivity (6) and importantly, reduces food intake (7,8). Regular treatment with the short acting GLP-1 receptor agonist, synthetic exendin-4 or exenatide, has been associated with improved diabetic control, reduced food intake and weight loss (9,10).

Blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (BOLD fMRI) allows visualisation of regional brain activation during task performance, by detecting changes in the oxygenation status of brain tissue in response to changes in neuronal activation (11). Conventional model-driven BOLD image data analysis requires task performance to be short and repeated, so that activation by the task is detected by signal change with the same periodicity as the stimulus that is presented. In investigations of appetite, the presentation of food images, alternating with non-food images of similar shapes and colors, has been successfully used to examine the brain's response to food cues (e.g. 12-14).

We set out to examine how the brain's response to food cues might be modulated by prior food ingestion and by an acute single exposure to exenatide in people with type 2 diabetes using BOLD fMRI. We also studied the effect of meal ingestion on responses to food cues, in young non-obese, non-diabetic subjects, without the exposure to drug.

Materials and Methods

Subjects: Study 1 (diabetic subjects): Twelve right-handed people, 4 female, mean (±SD) age 56.1±6.4 yrs; BMI 31.1±3.5kg/m^2; HbA1c 7.0±0.8%, with type 2 diabetes were recruited in primary care. Insulin resistant diabetes was inferred by recruiting people achieving glycated hemoglobin (HbA1c) <8.0% on lifestyle therapies alone (n=5) or on lifestyle plus metformin (n=7, dose range: 1-2g/day). People using insulin, insulin secretagogues, thiazolidinediones or incretin-based therapies; or with HbA1c ≥8%, BMI ≥35kg/m^2 or contraindications to fMRI (claustrophobia, metal implants, pacemakers, history of eye trauma) were excluded.

Study 2 (Young, lean, non-diabetic subjects): Twelve right-handed non-diabetic volunteers, 5 female, aged 26.3±4.2yrs; BMI 23.1±3.0kg/m^2; HbA1c 5.2±0.4% were studied in a similar protocol, in absence of study medication.

Protocol:

Study 1 (diabetic subjects): Patients undertook 4 sessions of fMRI scanning, at the Centre for Neuroimaging Sciences at King's College London's Institute of Psychiatry, using a 1.5 Tesla GE SIGNA HDx scanner (General Electric, Milwaukee, WI) equipped with echo-speed gradient coils (ANMR, Woburn MA). Study order was block randomised by the Clinical Trials Unit at the Institute, separated by at least one week. Studies were performed in the morning, after an overnight (8-10 hr)

fast, during which sips of water were permitted. An intravenous catheter was inserted using aseptic technique in a left antecubital vein. After 15 minutes rest, blood was drawn, followed by double-blind subcutaneous injection of 10mcg of exenatide (Eli Lilly, Basingstoke, Hampshire, UK) (active study) or an equal volume of saline (placebo) on two occasions each (t=-16min) in the anterior abdominal wall, administered by a research nurse. The subject was made comfortable supine on the scanner trolley with head position maintained by a moulded headrest. Earphones were worn to reduce exposure to scanner noise and to allow radiographers and investigators to speak to the subject. After localization, a 43-slice high-resolution image was acquired in the anterior commisure–posterior commisure plane (slice thickness 3mm; slice gap 0.3mm; matrix 128x128; Field of view 240; flip angle 90; TE=40ms; TR=3s) (t=-17min); followed baseline global cerebral blood flow measurement using continuous arterial spin labelling (slice thickness 3mm; matrix 128x128; flip angle 90; scanning time: 5.5 min) (14).

At -6 minutes, the subject was removed from the scanner and sat upright to consume a 554kcals mixed meal [36.8g fat (22.2g saturated); 7.8g protein and 47.6g carbohydrate], in a palatable and easily eaten form: 200ml softened ice cream (Häagen-Dazs Belgian chocolate ice cream, Uxbridge, Middlesex, UK) on 2 occasions (FED) or 50ml water on 2 occasions to mimic the physical actions of ingestion (FASTED). After repositioning the subject in the scanner (t=0min), a further localization scan was performed.

Subjects were asked to rate hunger (“How hungry are you feeling?”); satiety (“How full are you feeling?” and “How pleasant would it be to eat now?”) and nausea (“How sick are you feeling?”) each on a 1–10 visual analogue scale (VAS) projected onto the scanner screen, using a button box-driven cursor to score each question at -16, -8, +6, +14, +26 and +34 minutes (15). Blood was drawn (for

glucose and insulin assay) immediately before the subcutaneous injection and on completion of each questionnaire.

Between +16min and +26min, subjects viewed 72 images each of food and non-food related objects presented in a block design on the screen. Each block contained 12 images of the same category, each image presented for 3s with no inter-stimulus interval. Food blocks were preceded by the verbal instruction “Imagine you are eating the following foods” and object blocks by “Imagine you are using the following objects”. Each block lasted 72s and was followed by the hunger question before the next block (15,16). The images were from a library held by Prof Janet Treasure at the Institute, which had been rated for recognisability, pleasantness and appeal, visual complexity and color (16). One hundred and eight T2* weighted whole-brain MR images displaying BOLD contrast were acquired, each of 41 near-axial slices (repetition time [TR] 4s; echo time [TE] 40ms; slice thickness 2.4mm; slice gap 1mm; matrix size 64x64; field of view 24cm and flip angle 90).

Study 2 (Non-Diabetic): Subjects were studied twice in random order, for FED and FASTED studies, using the same protocol, but without any injection.

Biochemical analysis

Blood was collected for glucose into sodium fluoride/K3-EDTA, and for insulin in clot activator tubes, kept on ice until study end, then centrifuged for 10min at 4ºC. Plasma glucose was analysed using a glucose oxidase technique (Yellow Springs Glucose Analyser, Yellow Springs Inc, Arizona USA); serum for insulin was stored at -20^0 C until analysis using a two-site sandwich immunoassay (Siemens Healthcare Diagnostics Ltd, Surrey UK, interassay and intraassay coefficient of variation 2.6-5.9% and 3.2-4.6%). Failure of cannulation caused incomplete data collection in 5 of 12 diabetic subjects for glucose, and insulin samples were not obtained in all 4 studies in a further subject.

Statistical analyses of biochemical results and appetite questionnaire

Net incremental area under the curve (iAUC) was calculated using the trapezoidal method for glucose, insulin and questionnaire score responses for each visit in each subject. Baseline and iAUC data were assessed for normality using the Shapiro-Wilk test and Q-Q plots, and were analysed using repeated measures ANOVA or Friedman's two-way ANOVA by rank method, with post-hoc Student's t tests or Wilcoxon signed-rank tests (uncorrected for multiple comparisons) for Study 1 and with paired Student's t tests or Wilcoxon signed-rank tests for Study 2. For clarity, all results are presented as means and standard errors. Statistical analyses were performed using the Statistical Package for Social Sciences for Mac (SPSS 19.0, SPSS inc., Chicago, IL, USA).

Image Analysis

Baseline perfusion ASL data were pre-processed using FSL 4.1 Brain Extraction Tool (17) and SPM8 (Statistical parametric mapping 8, Wellcome Trust Centre for Neuroimaging at UCL) for brain extraction and normalisation to a standard EPI template respectively. The average global cerebral blood flow was extracted using Marsbarr (18) and compared using repeated measures analysis in SPSS.

BOLD fMRI data were analysed using the statistical package, XBAM version 4.1, applying non-parametric methods to minimise assumptions (http://brainmap.it) (19,20). The analyses included first construction of brain activation maps showing areas where BOLD signal increased or decreased significantly in response to food image viewing within each state (signal with food images greater or less than signal with non-food images) and then two-way comparisons between the placebo and exenatide treated state in each nutritional state (fed or fasted). To make these comparisons, data were processed to minimise motion related artefacts (20) and responses to the experimental paradigms detected by convolving with 4 and 8s Gamma variate functions, to model the BOLD response separately. A weight sum of the two convolutions that provided the best fit to the time series at each voxel was computed using a constrained BOLD effect model (21). A goodness of fit statistic was computed, as the ratio between the sums of squares of deviations from the mean image intensity (over the whole time series) due to the model, and the sum of squares of deviations due to the residuals (SSQratio). The data were then permuted by a wavelet-based method (22). A data-driven null distribution of SSQratios under the assumption of no experimentally determined response was formed by the results of repeated permutations and the critical value of SSQratio needed to threshold the map at any desired type or error rate calculated. The size of the BOLD response, the effect size, was also computed. The observed and permuted SSQratio maps and the BOLD effect size maps for each subject were transformed into standard space (23). A group generic brain activation map was

constructed by a cluster-level threshold that yielded 0.5 false positive cluster per map to the null distribution of SSQratios formed by the permuted data, which corresponded to cluster-level p-value in the range of 0.005-0.009 for all the analysed presented here. Comparisons between conditions were carried out by computing the difference in median effect size between measurements at each voxel and comparing them to a critical effect size derived from random wavelet-permutation of group membership: cluster-wise maps were then obtained using the same method as for group-level maps (19). Talairach Daemon (24) software was also used to determine the corresponding anatomical regions of detected voxels on the activation maps.

Results

Study 1 (diabetic subjects)

Glucose and insulin responses: Fasting plasma glucose was not significantly different between the four study arms (Friedman test, χ^2=4.547, *d.f.*=3, *p*=0.208) (figure 1a). There were no main or interaction effects of feeding status (F=0.856, *p*=0.391) or exenatide (F=0.096, *p*=0.768, interaction F=9.06, *p*=0.652) on iAUC(glucose) (figure 1b). Fasting insulin concentrations were also not different between studies (Friedman test χ^2=1.4, *d.f.*=3, *p* = 0.706,) (figure 1c). There were no main (F=0.038, *p*=0.853) or interaction effects (F=0.230, *p*=0.652) of feeding status on iAUC(insulin) but there was a trend towards greater iAUC(insulin) with exenatide (main effect F=5.779, *p*=0.061; estimated means (±S.E.) $iAUC(insulin)_{placebo}$=1.19±1.0mU·hr/L, $iAUC(insulin)_{exenatide}$=12.66±4.56mU·hr/L) (figure 1d).

Symptom Scores: Immediately post-exenatide/placebo injection, there were no significant differences in reported hunger (Friedman test χ^2=2.533, *d.f.*=3, *p*=0.469), (figure 2a) or fullness (Friedman test, χ^2=6.75, *d.f.*=3, *p*=0.08) (figure 2b) between the study arms. Exenatide elicited lower initial scores for pleasantness to eat (main effect, F=6.026, *p*=0.032; estimated means (±S.E.) placebo=4.8±0.7, exenatide=4.0±0.6) (figure 2c) and greater feelings of sickness (Friedman test, χ^2=8.789 *p*=0.032, *d.f.*=3, *p*=0.032; exenatide-FED=2.25±0.43 vs: placebo-FED=1.33±0.22, z=-2.070, *p*=0.038; and vs

placebo-FASTED=1.25±0.18, z=-2.271, *p*=0.023) (figure 2d). There were no subsequent differences in iAUC for hunger (feeding status, F=1.086, *p*=0.320; exenatide, F=1.111, *p*=0.314; interaction, F=0.010, *p*=0.890) fullness (feeding status, F=0.324, *p*=0.581; exenatide, F=0.176, *p*=0.683; interaction, F=0.750, *p*=0.405), or pleasantness to eat (feeding status, F=0.034, *p*=0.857; exenatide, F=1.879, *p*=0.198; interaction, F=0.809, *p*=0.388) or sickness scores (Friedman test, χ^2=2.9, *d.f.*=3, *p*=0.407) (figure 2e).

Neuroimaging data: There were no differences in baseline global cerebral blood flow in the four study arms (Interaction of food and drug, $p = 0.796$).

Figure 3 shows group maps of brain regions responding to food and non-food image viewing with change in BOLD signal. For clarity, every third slice from slices 9 to 30 (out of total 43) are displayed. The cluster information, number of voxels, the Talairach coordinates of the most activated voxel in each cluster and the Brodmann area for each region identified with a significant change in activation, both positive (food>non-food) and negative (non-food>food) are listed in supplementary data, table 1, while Table 1 lists similar data for the brain regions found to have significant differences in signal change between all four states, including the effect of exenatide.

Activation in response to food image viewing (food>non-food contrast, red in figure 3) in placebo-FASTED (Figure 3a) was detected in right lingual gyri, medial frontal gyri, middle frontal gyri, superior frontal gyrus, fusiform gyri, and bilateral cerebellum (Supplementary data, Table 1). In exenatide-FASTED (Figure 3b, red), the lingual gyri, right medial frontal gyrus, right middle/superior frontal gyrus, right inferior frontal gyrus and right insula were activated in response to food images. In placebo-FED (Figure 5c red), left medial frontal gyrus, left middle and superior frontal gyri including the dorsolateral prefrontal cortex (DLPFC) were activated (Supplementary data. Table 1). In exenatide-FED, no significant cluster was observed (Figure 3d).

Reduced activation in response to food image viewing (food<non-food) in placebo-FASTED (Figure 3(a) yellow) occurred in left inferior parietal lobule, bilateral insula, right putamen, left middle temporal gyrus, left parahippocampal gyrus, postcentral gyri, precentral gyri, sub-gyral, superior temporal gyri and transverse gyri. In exenatide-FASTED (figure 3(b) yellow), there was significant deactivation on food image viewing in bilateral cerebellum, left inferior parietal lobule, insula, middle temporal gyri, parahippocampal gyri, left postcentral gyrus, precentral gyri, left sub-gyral, superior temporal gyri, transverse temporal gyri, fusiform gyri, lingual gyri, left middle and superior frontal gyrus, middle occipital gyri, bilateral posterior cingulate, left precuneus and right supramarginal gyrus. In placebo-FED (Figure 3(c) yellow), left inferior parietal lobule, left postcentral gyrus, precentral gyri, left sub-gyral, bilateral superior parietal lobule, cingulate gyri, medial frontal gyri, bilateral paracentral lobule and bilateral precuneus were deactivated. In exenatide-FED (Figure 3(d) yellow), deactivation was seen in left inferior parietal lobule, left parahippocampal gyrus, postcentral gyri, precentral gyri, left middle/transverse temporal gyri, right middle frontal gyrus, left supramarginal gyrus, cingulate gyri, medial frontal gyri, bilateral paracentral lobule, bilateral precuneus, caudate.

The effect of exenatide on responses to food image viewing in the fasting and fed states: In the FASTED state, exenatide, compared to placebo, increased the effect size in right insula, right inferior frontal gyrus, right lentiform nucleus (putamen/lateral globus pallidus), right middle frontal gyrus and DLPFC, and right amygdala, as shown in red in figure 4a (slice 13-20). Cluster information is given in Supplementary data Table 2. This effect was caused by activation in drug and deactivation in placebo-FASTED.

In the FED state, exenatide, compared to placebo, reduced effect sizes in left inferior frontal gyrus, left middle frontal gyrus and DLPFC and left insula, shown in yellow in Figure 4b. Cluster information is given in Supplementary data Table 2. This reduction was caused by activation (signal change with

food>signal change with non-food image viewing) in placebo-FED and de-activation (signal change with food image viewing<signal change with non food image viewing) in exenatide-FED.

Study 2 (non-diabetic subjects)

Glucose and insulin responses: Baseline plasma glucose concentrations were not statistically different between studies (t=-0.498, p=0.628) (figure 5a), but rose within normal limits after the meal ($iAUC(glucose)_{fed}$=0.79±0.07mmol·hr/L, $iAUC(glucose)_{fasted}$=0.23±0.06mmol·hr/L, t=6.142, p<0.001) (figure 5b). Baseline insulin concentrations were also not statistically different between studies (z=-1.897, p=0.058) (figure 5c), but rose after the meal ($iAUC(insulin)_{fed}$=8.56±3.66mU·hr/L, $iAUC(insulin)_{fasted}$=-0.86±0.39mU·hr/L, z=-2.547, p = 0.011) (figure 5d).

Symptom Scores: At baseline there were no significant differences in any symptom scores (hunger, t=-1.595, p=0.139; fullness, t=0.944, p=0.365; pleasantness to eat, t=-0.583, p=0.571; sickness, z=-1.337, p=0.181) between the two arms (figure 6a-d). Feeding increased sensation of fullness ($iAUC(fullness)_{fed}$=1.46±0.36 VAS·hr, $iAUC(fullness)_{fasted}$=0.02±0.43 VAS·hr, t=2.434, p=0.038) (figure 6e), and reduced measures of pleasantness to eat ($iAUC(pleasant)_{fed}$=-0.70±0.36 VAS·hr, $iAUC(pleasant)_{fasted}$=0.40±0.33 VAS·hr, z=-2.395, p=0.017) (figure 6f), with a trend towards reduced hunger ($iAUC(hunger)_{fed}$=-0.67±0.45 VAS·hr, $iAUC(hunger)_{fasted}$=0.50±0.36 VAS·hr, t=-1.988, p=0.078) (figure 6a), but with no effect on sickness iAUC (z=-0.663, p=0.508)

Neuroimaging data: At baseline, there was no difference between the two arms of study 2 (main effect of food, p=0.901). Figure 7 shows the group maps of brain regions responding to the food and non-food imaging viewing stimulation with a change in BOLD signal: 7a for FASTED and 7b for FED, with every third slice from slice 9 to 30 displayed. Food, in contrast to non-food, images activated left lingual gyrus and left thalamus in FASTED (Figure 7a red) and right middle frontal gyrus, DLPFC and

right sub-gyrus in FED (Figure 7b red). In contrast, food images deactivated right insula and left superior temporal gyrus in FASTED (Figure 7a yellow) and right superior temporal gyrus and left middle temporal gyrus in FED (figure 7b yellow) (Supplementary data: Table 2).

DISCUSSION

This study examined the effect of feeding and of a single exposure to a GLP-1 receptor agonist on the brain's responses upon viewing food images, compared to non-food related object images, in both the fasted and fed states in people with insulin resistant type 2 diabetes. The anatomy of responses was delineated using BOLD fMRI, in which changes in magnetic signal due to changes in regional oxygenation status are imaged. Changes in BOLD signal thus reflect changes in the regional brain perfusion, as a surrogate marker of neuronal activation. The main finding is an impact of the GLP-1 receptor agonist to increase activation by food image viewing of reward pathways such as lentiform nucleus and amygdala in the FASTED state and decrease activation in brain areas involved in modulation of executive function, such as DLPFC, in the FED state in people with type 2 diabetes. It was also noteworthy that feeding had no impact on fullness and hunger scores in diabetic volunteers and although exenatide did not have significant impact on this, it did reduce the desire to eat immediately after injection, seen as reduced scoring of the item "pleasantness to eat now".

The brain regions differently activated by food image viewing with exenatide in the fasted state in our subjects included right lentiform nucleus, part of the dorsal striatum (associated with encoding food reward during eating and modulated by the feeling of appetite (25-29)) and amygdala, part of the dopaminergic limbic reward network (30). The insula, where exenatide also altered responses to food image viewing, has been associated with processing information related to taste and hedonic evaluation of food (27). An impact of exenatide on these regions' responses to food cues in the fasted state is compatible with reduced drive to initiate eating.

Brain regions affected by exenatide in response to food cues in the fed state in type 2 diabetes included regions known to be active in voluntary modulation of behaviour (DLPFC, left inferior frontal gyrus and middle frontal gyrus, (31)). The DLPFC provides inhibitory executive function, limiting reward-generating mechanisms. It receives input from the cortico-limbic system and is involved in maintenance and monitoring of eating-related goals and modulation of reward and satiation responses. An impact of exenatide on its function is compatible with reduced drive to eat more in the fed state (32).

In support of our interpretation of the neuroimaging data, diabetic subjects showed no change in satiety and fullness scores in response to meal ingestion. This was in marked contrast to the results of our study in young, non-diabetic volunteers, where meal ingestion was followed by changes in subjective measures of satiation and activation of the DLPFC and prefrontal cortex in response to food image viewing in the fed state only.

In human studies, peripheral GLP-1 and GLP-1 analogue infusions are associated with satiety and early meal termination in fed individuals (32) and reduced food intake in fasted subjects offered a meal (33). In animal studies, intracerebroventricular (ICV) administration of GLP-1 and its receptor agonists result in meal termination in fed animals, suggesting at least part of the appetite suppressant effects of GLP-1 are centrally mediated (34). Confirmatory studies show increased food intake in satiated rats given the GLP-1 receptor antagonist Exenedin (9-39),_Ex9, both peripherally (35) and centrally. Williams et al demonstrated that ICV EX9 reversed the inhibitory feeding effect of ICV GLP-1 but had little effect on anorexia induced by peripheral GLP-1 infusion (36). These data suggest that GLP-1 acts on neuronal GLP-1 receptors to cause decreased food intake independent of peripheral GLP-1 action. Our data are compatible with this and suggest a neuroanatomical basis for the GLP-1

effect on eating behaviour in people with diabetes, of relevance not just to our understanding of the actions of GLP-1 on appetite control, but also to the understanding of eating behaviour more generally. Our data are also compatible with a positron emission tomography study, which showed association between peak postprandial plasma GLP-1 concentrations with increases in regional cerebral blood flow in the left DLPFC. Neither study allows us to determine whether the GLP-1 action is a direct effect on blood flow in this region or an indirect effect of neuronal and glial activation in the region, driven by the activation of the GLP-1 receptors, causing a secondary increase in regional blood flow. The latter would fit with the behavioural changes described for GLP-1 receptor activation and is the conventional interpretation of the neuroimaging data.

A food-imaging study found obese non-diabetic children to have greater left DLPFC activation when viewing food images than normal weight children (37). Although this was suggested to be associated with a need for greater result of self-control (31,38), our data suggest an alternative explanation, namely that in the inferred insulin resistant state (the obese children and our subjects with type 2 diabetes), food image viewing is associated with a desire to eat, even, as in our case in the fed state, which may be driving eating behaviour inappropriately. If so, this regional brain effect may provide a causative link between insulin resistance and obesity. In the present study, we found exenatide did reduce scores ranking desire to eat at baseline. We did not find any effect on the later responses, after meal or water ingestion, but the study is small and the variance in these assessments may have mitigated against detecting an effect.

Our study is limited by relatively small subject numbers of mixed gender. Women have a lesser response to food cues, which may have reduced our ability to detect an effect of food or subsequently a modifying effect of drug in affected brain regions. Similarly, some of our effects may have been driven by the taste or chemical composition of the chocolate in the ice-cream as flavinoids, found in

chocolate, may have direct effects on cortical function. However, our study gains strength because of the strict control of the fed versus fasted state of the subjects and our subsequent ability to examine the effect of feeding on the brain's response to the subsequent food cues and the effect of the drug differently in the fed and fasted state. Finally, we cannot seek an effect of diabetes per se as the non-diabetic subjects in study two, done to describe the normal responses to our meal challenge are not a comparable group.

In conclusion, we have shown an effect of the GLP-1 receptor agonist exenatide, to alter the neuronal responses of to food cues in both the fasted and the fed state, which may explain the agent's action on food intake and subsequent body weight and diabetes control in people with type 2 diabetes through allowing more effective self-control and self-monitoring in food intake. Further examination of the identified pathways may support the development of other new agents to address appetite control, obesity and type 2 diabetes in future.

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References

1. Roglic G, Unwin N, Bennett PH, Mathers C, Tuomilehto J, Nag S, Connolly V, King H. The burden of mortality attributable to diabetes: realistic estimates for the year 2000. *Diabetes Care* 2005;**28**:2130-35

2. Sjöstrand M, Eriksson JW. Neuroendocrine mechanisms in insulin resistance. *Mol Cell Endocrinol* 2009;**297**:104-11

3. Holst JJ, Vilsbøll T, Deacon CF. The incretin system and its role in type 2 diabetes mellitus. *Mol Cell Endocrinol* 2009;**297**:127-36

4. Holst JJ. Glucagon and glucagon-like peptides 1 and 2. *Results Probl Cell Differ* 2010;**50**:121-35

5. Toft-Nielsen MB, Damholt MB, Madsbad S, Hilsted LM, Hughes TE, Michelsen BK, Holst JJ. Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. *J Clin Endocrinol Metab* 2001;**86**:3717-23

6. Edholm T, Degerblad M, Grybäck P, Hilsted L, Holst JJ, Jacobsson H, Efendic S, Schmidt PT, Hellström PM. Differential incretin effects of GIP and GLP-1 on gastric emptying, appetite, and insulin-glucose homeostasis. *Neurogastroenterol Motil* 2010;**22**:1191-200

7. Gutzwiller JP, Göke B, Drewe J, Hildebrand P, Ketterer S, Handschin D, Winterhalder R, Conen D, Beglinger C. Glucagon-like peptide-1: a potent regulator of food intake in humans. *Gut* 1999;**44**:81-6

8. Gutzwiller JP, Drewe J, Göke B, Schmidt H, Rohrer B, Lareida J, Beglinger C Glucagon-like peptide-1 promotes satiety and reduces food intake in patients with diabetes mellitus type 2. *Am J Physiol* 1999;**276**:R1541-4

9. Davies MJ, Donnelly R, Barnett AH, Jones S, Nicolay C. Kilcoyne A. Exenatide compared with long-acting insulin to achieve glycaemic control with minimal weight gain in patients with type 2 diabetes: results of the Helping Evaluate Exenatide in patients with diabetes compared with Long-Acting insulin (HEELA) study. *Diabetes, obesity and metabolism* 2009;**11**:1153-62

10. Norris SL, Lee N, Thakurta S, Chan BKS. Exenatide efficacy and safety: a systematic review. *Diabetic Medicine* 2011;**26**:837-46.

11. Jezzard P, Buxton RB. The clinical potential of functional magnetic resonance imaging. *J Magn Reson Imaging* 2006;**23**:787-93

12. Führer D, Zysset S, Stumvoll M. Brain activity in hunger and satiety: an exploratory visually stimulated FMRI study. *Obesity (Silver Spring)* 2008;**16**:945-50

13. Frank S, Laharnar N, Kullmann S, Veit R, Canova C, Hegner YL, Fritsche A, Preissl H. Processing of food pictures: influence of hunger, gender and calorie content. *Brain Res.* 2010;**1350**:159-66

14. Dai, Garcia, de Bazelaire and Alsop, MRM 2008: 60: 1488-1497

15. Goldstone AP, Prechtl de Hernandez CG, Beaver JD, Muhammed K, Croese C, Bell G, Durighel G, Hughes E, Waldman AD, Frost G, Bell JD. Fasting biases brain reward systems towards high-calorie foods. *Eur J Neurosci.* 2009;**30**:1625-35

16. Uher R, Treasure J, Heining M, Brammer MJ, Campbell IC. Cerebral processing of food-related stimuli: effects of fasting and gender. *Behav Brain Res* 2006;**169**:111-19

17. Smith SM. Fast robust automated brain extraction. *Human Brain Mapping* 2002;**17**:143-55.

18. Brett M, Anton J-L, Valabregue R, Poline J-B. Region of interest analysis using an SPM toolbox. *8th International conference on functional mapping of the human brain* 2002; **16**.

19. Brammer MJ, Bullmore ET, Simmons A, Williams SCR, Grasby PM, Howard RJ, Woodruff PMR, Rabe-Hesketh S. Generic brain activation mapping in fMRI: a nonparametric approach. *Magn. Res. Imag.* 1997;**15**:763-770

20. Bullmore ET, Brammer MJ, Rabe-Hesketh S, Curtis V, Morris RG, Williams SCR, Sharma T, McGuire PK. Methods for diagnosis and treatment of stimulus-correlated motion in generic brain activation studies using fMRI. *Human Brain Mapping* 1999;**7**:38-48

21. Friman O, Borga P, Lundberg P, Knutsson H. Adaptive analysis of fMRI data. *NeuroImage* 2003;**19**:837-845

22. Bullmore ET, Long C, Suckling J, Fadili J, Calvert GA, Zelaya F, Carpenter TA, Brammer MJ. Coloured noise and computational inference in neurophysiological (fMRI) time series analysis: resampling methods in time and wavelet domain. *Human Brain Mapping* 2001;**21**:61-78

23. Talairach J, Tournoux P. *Co-planar stereotaxic atlas of the human brain*. Thieme, New York 1998.

24. Lancaster JL, Woldorff MG, Parsons LM, Liotti M, Freitas CS, Rainey L, Kochunov PV, Nickerson D, Mikiten SA, Fox PT. Automated Talairach Atlas labels for functional brain mapping. *Human Brain Mapping* 2000;**10**:120-131

25. Bohon C, Stice E, Spoor S. Female emotional eaters show abnormalities in consummatory and anticipatory food reward a functional magnetic resonance imaging study. *Int J Eat Disord* 2009;**42**:210-21

26. Small DM, Jones-Gotman M, Dagher A. Feeding-induced dopamine release in dorsal striatum correlates with meal pleasantness ratings in healthy human volunteers. *NeuroImage* 2003;**19**:1709-15

27. Small DM. Taste representation in the human insula. *Brain Struct. Func.* 2010;**214**:551-61

28. Stice E, Spoor S, Bohon C, Valdhuizen MG, Small DM. Relation of reward from food intake and anticipated food intake to obesity: a functional magnetic resonance imaging study. *J. Abnorm Psychol.* 2008;**117**:924-35

29. Volkow ND, Wang GJ, Maynard L, Jayne M, Flower JS, Zhu W, Logan J, Gatley SJ, Ding YS, Wong C, Pappas N. Brain dopamine is associated with eating behaviours in humans. *Int J Eat Disord* 2003;**33**:136-42

30. Berthoud H-R, Lenard NR, Shin AC. Food reward, hyperphagia, and obesity. *Am J Physio Regul Integr Comp Physiol* 2011;**300**:R1266-77

31. Alonso-Alonso M. Brain imaging, the prefrontal cortex, and obesity: where do we stand? *Obesity and weight management* 2010;**6**:126-30

32. Ahren B, Holst JJ, Mari A. Characterization of GLP-1 effects on beta-cell function after meal ingestion in humans. *Diabetes Care* 2003;**26**:2860-4

33. de Graaf C, Blom WAM, Smeets PAM, Stafleu A, Hendriks HFJ. Biomarkers of satiation and satiety. *American Journal of clinical nutrition* 2004;**79**:946-61

34. Tang-Christensen M, Larsen PJ. Central administration of GLP-1-(7-36) amide inhibits food and water intake in rats. *Am J Physiol* 1996;**271**:R848-56

35. Meeran K, O'Shea D, Edwards CM, Turton MD, Heath MM, Gunn, I, Abusnana S, Rossi M, Small CJ, Goldstone AP, Taylor GM, Sunter D, Steere J, Choi SJ, Ghatei MA, Bloom SR. Repeated intracerebroventricular administration of glucagon-like peptide-1-(7-36) amide or exendin-(9-39) alters body weight in the rat. *Endocrinology* 1999;**140**:244-50

36. Williams DL, D. G. Baskin DG, Schwartz MW. Evidence that intestinal glucagon-like peptide-1 plays a physiological role in satiety. *Endocrinology* 2009;**150**:1680-7

37. Davids S, Lauffer H, Thoms K, Jagdhuhn M, Hirschfeld H, Domin M, Hamm A, Lotze M. Increased dorsolateral prefrontal cortex activation in obese children observation of food stimuli. *Int. J. Obesity* 2010;**34**:94-104

38. Gottfried JA, O'Doherty J, Dolan RJ. Encoding predictive reward value in human amygdala and orbitofrontal cortex. *Science* 2003;**301**:1104-7

Figure Legends

Figure 1. Plasma glucose and insulin responses for study one, subjects with type 2 diabetes. (a) Plasma glucose plotted against time. Dotted line = fasted + placebo study; dash-dot line = fasted + exenatide ; solid line = fed + placebo; and dashed line = fed + exenatide (b) iAUC for plasma glucose (diagnonal hatched bar = fasted + placebo; vertical hatch fasted + drug; stippled bar = fed + placebo; open bar fed + exenatide. Panels (c and d) show the insulin responses, with the same key.

Figure 2: Visual analogue scales for study one (subjects with type 2 diabetes). Symbols as in Figure 1a. Panel (a) = hunger score; panel (b) Fullness score; panel (c) Pleasantness score and panel (d) sickness score. Panel (e) shows iAUC for sick score (placebo fasted; drug fasted; placebo fed; drug fed)

Figure 3: Activation maps for study 1, subjects with type 2 diabetes (a) fasted + placebo (b) fasted + drug (c) fed + placebo and (d) fed + drug fed. Red: food image viewing > non-food image viewing; Yellow: non-food image viewing > food-image viewing; all $p < 0.009$

Figure 4: ANOVA analysis for study 1, subjects with type 2 diabetes, comparing exenatide and placebo (a) in the fasted state (b) in the fed state. Red indicates regions with significant increase in effect size with exenatide vs placebo; yellow, significantly less effect with exenatide vs placebo; all $p < 0.009$.

Figure 5: Plasma glucose and insulin responses for study two, lean non-diabetic subjects.

Panel (a) shows the plasma glucose plot, dashed line = for fasted and solid line = fed studies; (b) shows the iAUC for plasma glucose (vertical hatched bar = fasted; diagonal hatched bar = fed) (c) Plasma insulin plot (d) iAUC for plasma insulin (symbols as for a and b)

Figure 6: Visual analogue scores for Study 2, non-diabetic group. Panel (a) hunger; panel (b) fullness; panel (c) pleasantness and panel (d) sickness. Key as for Figure 2.

Figure 7: Activation maps for Study 1, lean, non-diabetic subjects (a) fasted (b) fed. Red: food image viewing > non-food image viewing; Yellow: non-food image viewing > food-image viewing, all $p < 0.009$

Table 1

Study 1 Type 2 diabetes Group: Brain regions shown significance difference in condition comparisons

Cerebral region	Brodmann's area	side	No. of voxels	Probability	Coordinates of most active voxel Tal (x)	Tal (y)	Tal (z)
Drug > Placebo in the fasted state							
Inferior frontal gyrus	47	R	205	0.005330	40	15	-3
Placebo > Drug in the fasted state							
No statistically significant cluster was detected.							
Drug > Placebo in the fed state							
No significant cluster found.							
Placebo > Drug in the fed state							
Middle frontal gyrus	10	L	173	0.005452	-31	47	9

Figure 1.

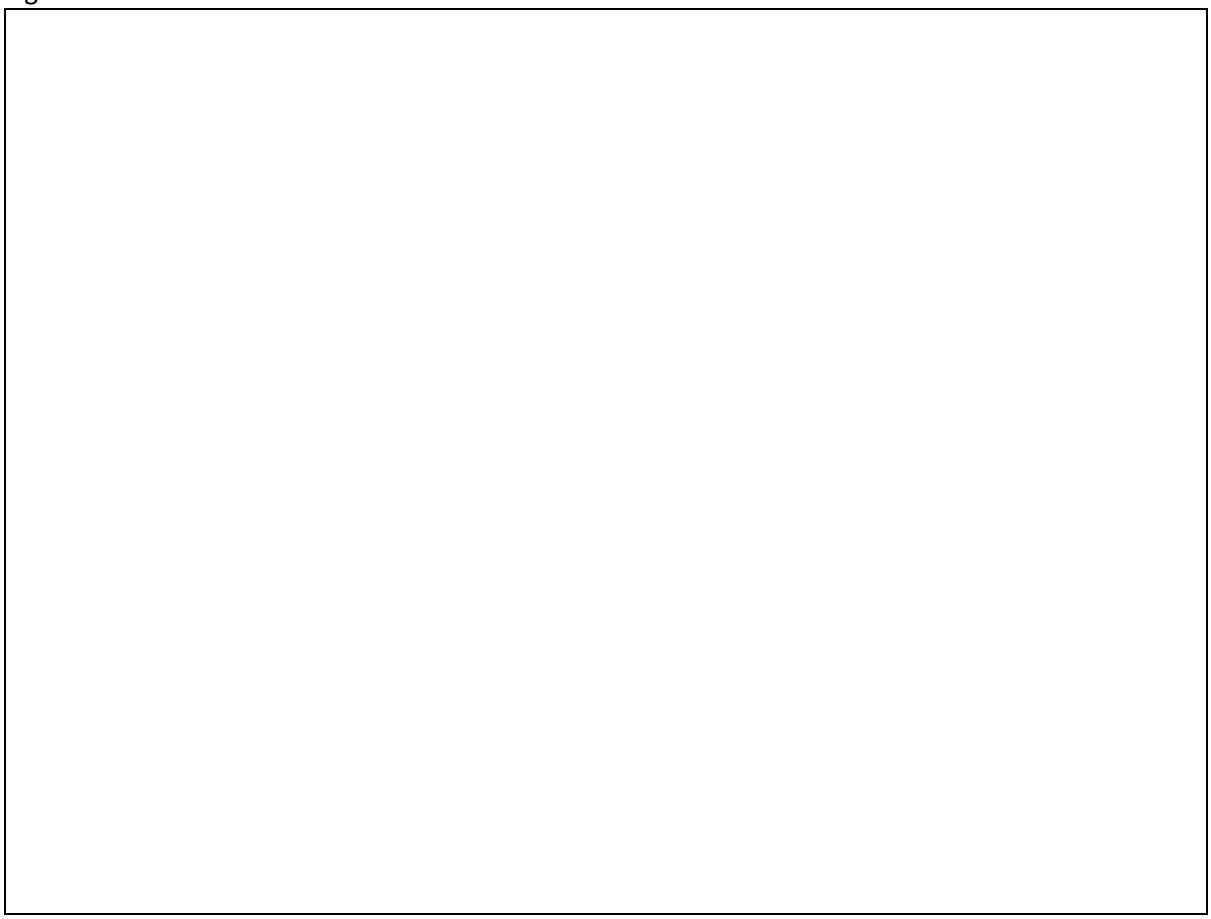

Figure 2 Study 1, VAS for Type 2 diabetes group

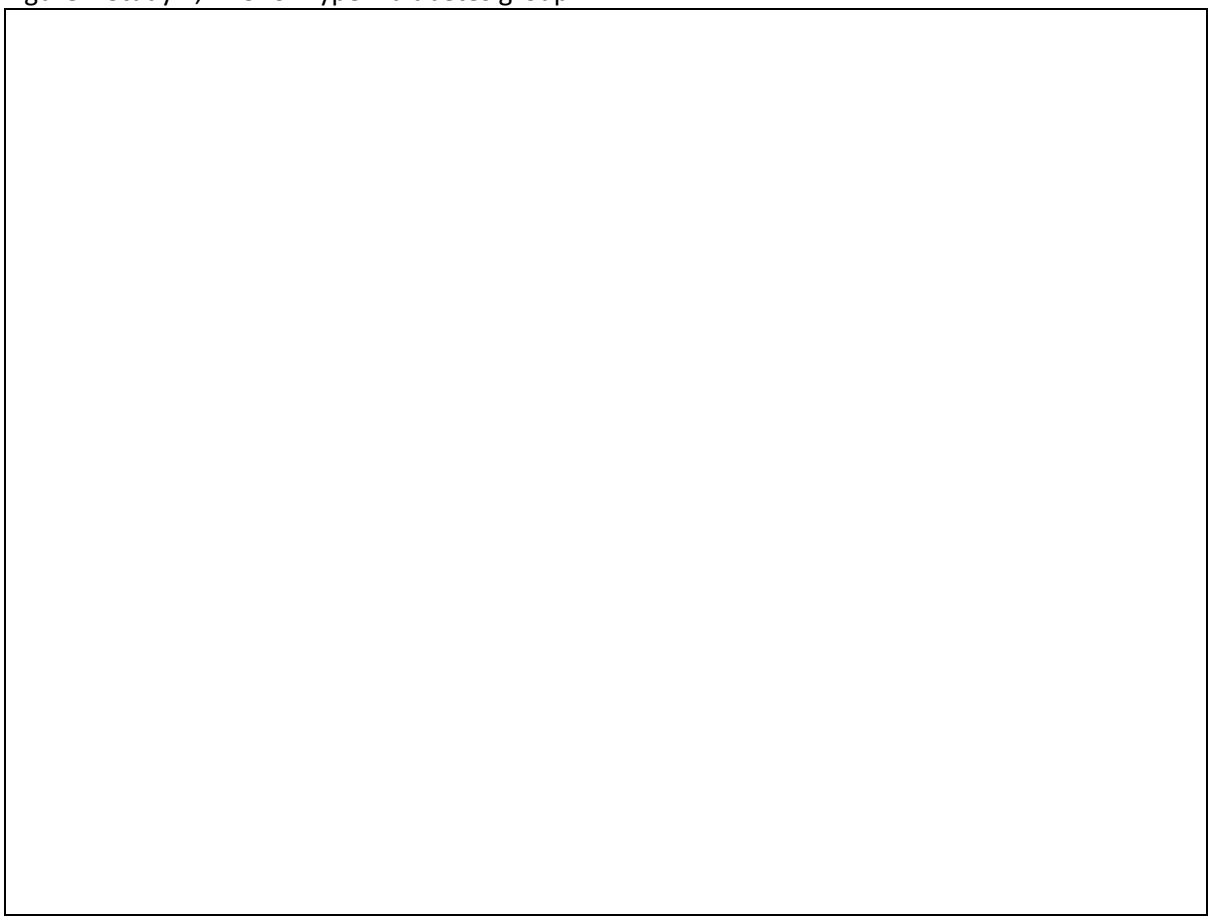

Figure 3

Figure 4.

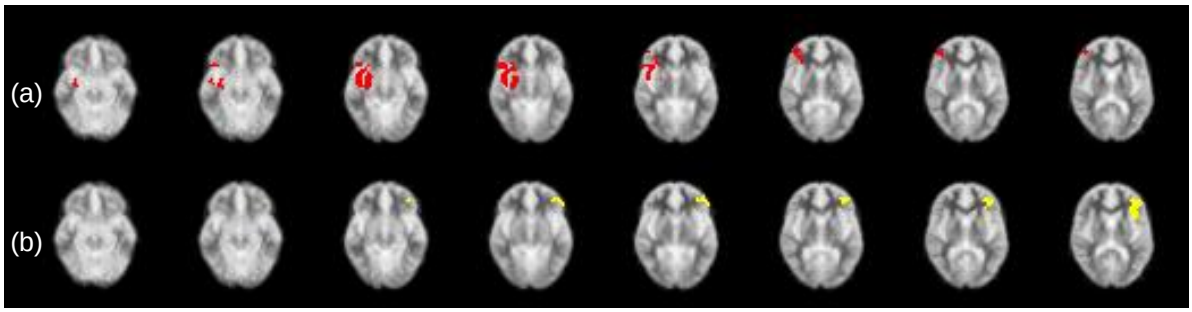

Figure 5

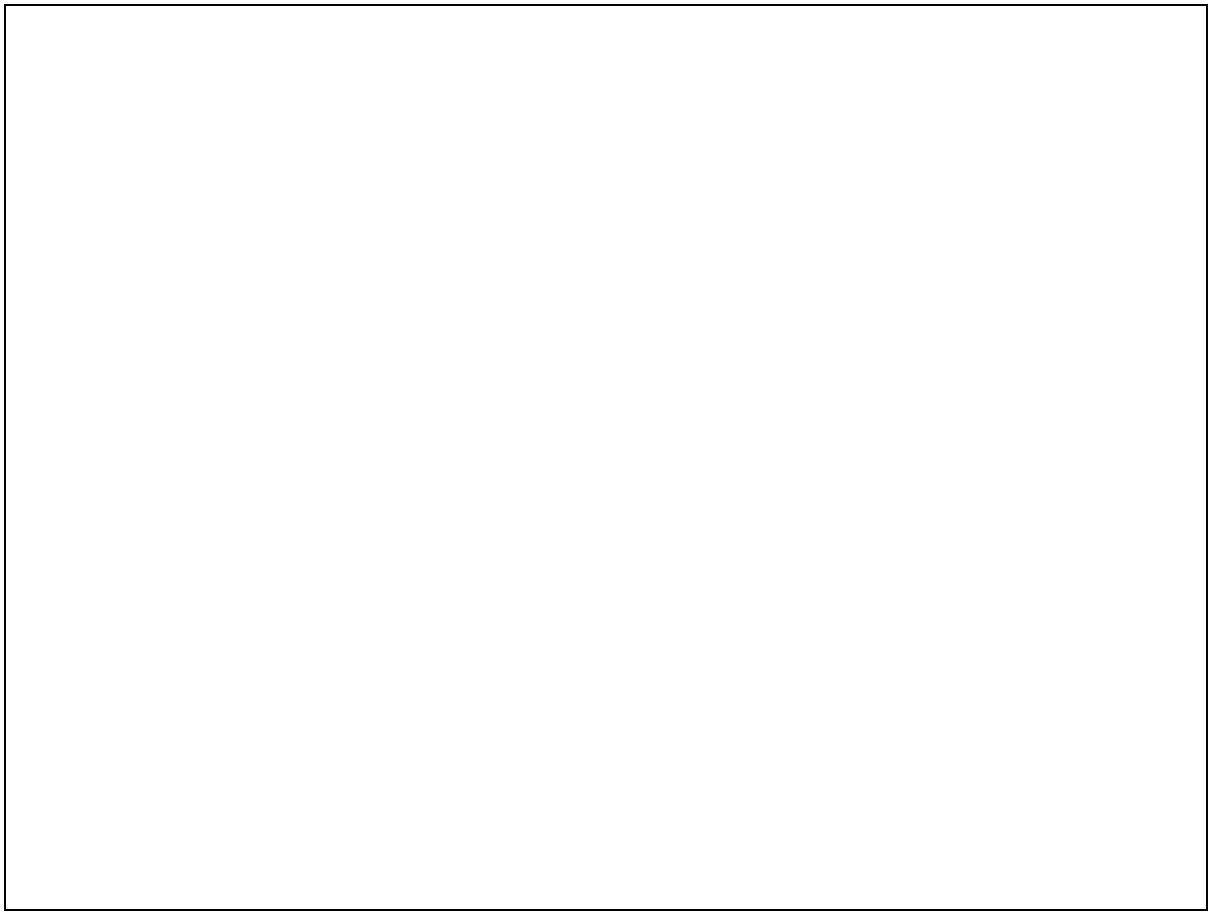

Figure 6

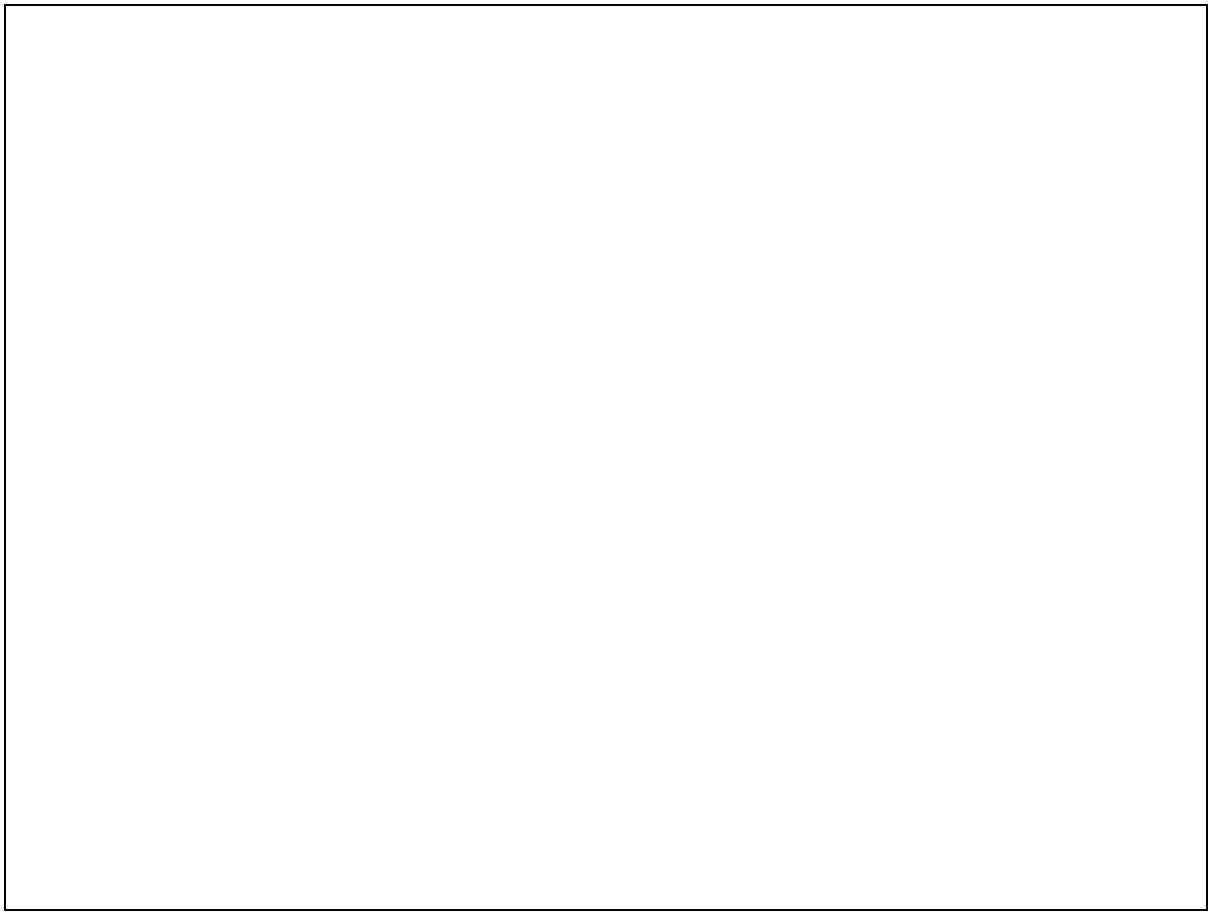

Figure 7