Weight loss induced increase in fasting ghrelin concentration is a predictor of weight regain: evidence from the Diabetes Remission Clinical Trial

Running title: Appetite-related predictors of weight regain in DiRECT

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Aim: To investigate whether appetite-related hormones were predictors of weight regain in the Diabetes Remission Clinical Trial (DiRECT).

Materials and methods: DiRECT is a cluster-randomised clinical trial designed to assess the effect of weight-loss on type 2 diabetes remission. For this *post hoc* analysis, data were available for 253 (147 interventions, 106 controls) individuals with type 2 diabetes (aged 53.6±7.5 years, BMI 34.7±4.4 kg/m^2, 59% males). Intervention participants received a 24-month weight-management programme and controls remained on usual diabetes care. Fasting plasma concentrations of leptin, ghrelin, GLP-1, and PYY were measured at baseline, 12 and 24-months in all participants, and at 5-months in a subset of interventions (n=56) and controls (n=22). Potential predictors were examined using multivariable linear regression models.

Results: The intervention group lost 14.3±6.0% body-weight at 5-months but regained over time, with weight-losses of 10.0±7.5% at 12-months and 7.6±6.3% at 24-months. Weight-loss in controls was 1.1±3.7% and 2.1±5.0% at 12 and 24-months, respectively. Body-weight increased by 2.3% [95% CI: 0.4,4.1]; p=0.019) between 12 and 24-months for every 1 ng/ml increase in ghrelin between baseline and 12-months, and weight regain between 12 and 24-months was increased by 1.1% (95% CI: 0.2,2.0; p=0.023) body-weight for every 1 ng/ml increase in ghrelin at 12-months.

Conclusion: The rise in ghrelin (but not any other measured hormone) during diet-induced weight-loss was a predictor of weight regain during follow-up, and concentrations remained elevated over time, suggesting a small but significant compensatory drive to regain weight. Attenuating the effects of ghrelin may improve WLM.

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Introduction

Despite expert clinical guidelines and numerous drug therapies, type 2 diabetes substantially reduces life expectancy (1) and is arguably the biggest concern with regards to the increasing prevalence of overweight and obesity, which has become the norm in Western societies (2). Encouragingly, 12-month results from the Diabetes Remission Clinical Trial (DiRECT) demonstrated that short duration (<6 years) type 2 diabetes is reversible in 64% of people who achieve weight-losses of 10kg (3), but clinical and economic benefits of remission are dependent almost entirely on weight-losses being maintained (3, 4). Significant weight-loss is possible across a range of dietary approaches (5), but the majority of people tend to regain weight over time (6, 7), and in DiRECT we observed an average weight regain of 44% in intervention participants between 5 and 24 months. Although weight-losses remained above average for a behavioural intervention, rates of remission were reduced from 46% at 12-months to 36% at 24-months (8).

Long-term weight-loss maintenance is the most difficult problem to tackle in obesity management (9). Weight regain after diet-induced weight-loss is considered to have a strong biological basis (10, 11), and there appears to be distinct physiological differences in the mechanisms regulating appetite, depending on whether an individual is at usual body weight or maintaining weight-loss. The homeostatic control of food intake occurs primarily within the arcuate nucleus of the hypothalamus, resulting from integration of hormonal signals from the gastrointestinal tract (e.g. ghrelin, GLP-1, PYY) and adipose tissue (e.g. leptin) with each conveying information regarding hunger, satiety and adiposity stores (12). It was reported in a landmark trial by Sumithran et al that diet-induced weight-loss of 14% body weight was associated with significant increases in the hunger hormone ghrelin and a reduction in satiety

promoting peptides (e.g. GLP-1, PYY), as well as increases in subjective appetite, changes which persisted up to 1-year in the context of weight regain (13). These findings have been widely interpreted as compensatory mechanisms encouraging weight regain, however this conclusion is somewhat speculative given that correlations between altered appetite hormones and weight regain were lacking in this study (13) and others (14-16). It is clear that appetite is a strong biological driver of eating, or not eating (17) but whether appetite hormone changes are simply a consequence of weight-loss, or a compensatory response opposing the maintenance of lost weight requires further investigation in studies with larger sample sizes (18). The objective of this *post-hoc* analysis of the DiRECT cohort was to investigate whether baseline, post weight-loss, and within trial changes of several key appetite-related hormones (fasting leptin, ghrelin, GLP-1, and PYY) were predictors of weight regain.

Subjects and methods

DiRECT was a cluster-randomised, clinical trial conducted within routine primary care practice. Ethical approval was obtained from the West of Scotland Research Ethics Committee (reference number: 13/WS/0314), and all participants provided written informed consent. The trial was registered at Controlled-Trials www.controlled-trials.com/ISRCTN03267836. The primary aim of the study was to assess the effect of weight-loss on type 2 diabetes remission, with a target weight-loss of 15kg. The main inclusion criteria were type 2 diabetes diagnosed <6 years, aged between 20-65 years, and body mass index of 27-45kg/m^2. Participants were not recruited if they had achieved weight-losses of >5kg in the last 6 months or had serious health problems (e.g. cancer, advanced kidney disease). The study protocol (19), recruitment and baseline data (20) and primary outcome results have all been published (3, 8).

GP practices were randomised to intervention or control, and intervention participants received the Counterweight-Plus weight management programme (21) delivered in their own GP practice by the practice nurse or local dietitian. Briefly, weight-loss was initiated by 'Total Diet Replacement' which provided 825-853 kcal/day of liquid formula diet (shakes/soups) for between 12-20 weeks during which time participants attended their practice nurse or dietitian for fortnightly review. This was followed by reintroduction of food, which involved replacing soups/shakes with calorie controlled meals and snacks over a 6-8 week period to meet energy balance requirements. Monthly visits to support long term WLM were ongoing to 24-months. Intervention group participants stopped all oral antidiabetes and antihypertensive drugs on commencing the weight management programme. Diabetes management continued as per current best practice clinical guidelines for control participants.

Data collection

For the purposes of this secondary analyses, weight (kg) and appetite hormone data from the DiRECT database were obtained at baseline, 5, 12 and 24-months. Baseline, 5-months (subgroup only), and 12-month fasting concentrations of plasma leptin, ghrelin, GLP-1 and PYY and weight changes were examined to identify potential predictors of WLM at 12 and 24-months in the majority of participants in the DiRECT study, and within a subgroup for whom biochemical data was available at 5-months. The blood samples that were available at 5-months for the study subgroup (56 intervention, 22 control) were obtained during food reintroduction after the intensive low-calorie diet period had ended, and were collected primarily for detailed metabolic studies to understand the mechanisms leading to type 2 diabetes remission, results of which have been published (22).

Appetite hormone measurements

Venous blood samples were collected into EDTA vacuette tubes, centrifuged (4 ^{o}C, 2000g for 15 min), and stored at -80^{o}C until analyses. Samples obtained from the Tyneside subgroup at 5-months had previously been defrosted and re-frozen on one occasion. Fasting plasma leptin, total ghrelin, total GLP-1 and total PYY were measured using the Meso Scale Discovery human metabolic U-PLEX group assay (MSD, Rockville, MD), a multiplex assay kit, which uses electrochemiluminescence detection technology to simultaneously quantify hormone concentrations. All assays were performed according to manufacturers' instructions. The median lower limits of detection of the assays was calculated as 0.65, 1.25, 0.15, and 0.49 pg/mL for leptin, ghrelin, GLP-1, and PYY, respectively. An external quality control sample was run in duplicate on each plate to determine intra and inter assay variation. The intra and inter assay coefficient of variation were 8.8% and 12.0% for ghrelin, 8.0% and 9.9% for GLP-1, 6.0 and 6.5% for PYY, and 6.1% and 14.3% for leptin."

Statistical analysis

In this exploratory analysis, appetite hormones measured at baseline and 1-year, and changes in their concentration from baseline to 5-month in the subgroup, and baseline to 12-months in all participants were used to predict weight change. Weight change at the following time-points was evaluated: baseline to 12-months, baseline to 24-months, 5 to 12-months, 5 to 24-months and 12 to 24-months. Weight change during follow-up has been assessed from 5-months onwards because weight-losses peaked around the end of TDR in the intervention group. Changes in weight and appetite hormones were assessed by Wilcoxon signed rank tests, and differences between intervention and controls by Mann-Whitney Wilcoxon tests. Potential predictors of weight regain were investigated using multivariable linear regression models adjusting for baseline weight, age, sex, treatment group (intervention or control) and the stratification variables practice list size ($\leq$ 5700, > 5700) and study centre (Scotland, Tyneside) and a random effect for practice. Models predicting weight change for study

periods starting after baseline additionally adjust for weight change from baseline to the start of the study period analysed (e.g. models predicting weight change from 5 to 12 months adjust for weight change from baseline to 5 months). Statistical significance was set at $p<0.05$. Since this is an exploratory analysis, P-values are not adjusted for multiple testing. All statistical analyses have been carried out using R version 3.6.2.

Results

Participants

Participant characteristics for the DiRECT study have been published previously (20). In this separate analysis, 253 participants (aged 53.6±7.5 [mean±SD], BMI 34.7±4.4 kg/m^2, 59% male) were included (n=147 interventions, n=106 controls) and summary characteristics are reported in Table 1. Blood sample data were available for 243 participants at baseline (n=144 interventions, n=99 controls), 219 participants at both baseline and 12-months (n=121 interventions, n=98 controls) and 201 participants at both baseline and 24-months (n=111 interventions, n=90 controls). Blood samples were also available at 5-months for a subgroup of participants (n=56 intervention, n=22 controls).

Changes in body weight and appetite-related hormones

Body weight

For all participants, mean weight change is displayed within Figure 1 and individual variability in weight change is shown in Figure 2. There were no significant differences in body-weight between intervention and control groups at baseline (p=0.470) but differences in weight-change from baseline to 12 and 24-months were highly significant ($p<0.001$). Mean (SD) body-weight change at 5-months in the intervention group (n=128) following total diet replacement and food reintroduction was 14.4±6.8kg/14.3±6.0% ($p<0.001$). Weight regain (n=123) between 5 and 12-months was 3.4±4.7kg/4.1±5.6% ($p<0.001$), and

6.4±5.8kg/7.7±6.8% (p<0.001) between 5 and 24-months, representing a regain of 24% and 44% of the initial body weight-loss, respectively. Overall, in the intervention group weight-loss at 12-months was 10.1±8.0kg/10.0±7.6% (p<0.001) with a mean weight regain of 2.6±5.1kg/3.1±5.6% (p<0.001) between year 1 and 2. On average, control participants lost 1.1±3.6kg/1.1±3.7% (p=0.003) at 12-months and 2.1±5.2kg/2.1±5.0% (p<0.001) at 24-months.

Appetite-related hormones

Baseline and within-trial changes in fasting appetite hormones are displayed in Figure 1 for the intervention and control groups. At baseline there were no significant differences in any of the plasma hormone concentrations between intervention and control groups. For intervention participants, weight-losses at 12 and 24-months were associated with significant reductions in leptin (12m, p<0.001; 24m p=0.002) and GLP-1 (p<0.001 at 12 and 24m), and ghrelin increased (p<0.001 at 12 and 24m) in comparison to baseline. The reduction in PYY was not significant at 12 (p=0.057) or 24-months (p=0.428). In the control group, at 12 and 24-months leptin did not change significantly from baseline but increases were observed in ghrelin (p<0.001), PYY (p<0.001) and GLP-1 (p<0.001). In the intervention group, leptin increased (p=0.011) between 12 and 24-months in correlation with weight regain, and in controls ghrelin increased (p=0.003) in association with a small mean weight-loss (1.0±4.2kg/1.0±4.2%; p=0.038) but other peptide levels remained stable. Significant differences in GLP-1, PYY and leptin were observed between intervention and controls at 12 and 24 months whereas statistical differences in ghrelin concentration were not evident at 12 (P=0.072) or 24 months (P=0.784). Baseline concentrations and within trial hormone changes did not have any associations with change in glycaemic control or diabetes remission status (data not shown).

Subgroup analyses

In the intervention subgroup, weight-loss at 5-months was associated with significant reduction in fasting plasma concentration of leptin ($p<0.001$) and GLP-1 ($p<0.001$) and significant increase in ghrelin ($p=0.002$) but PYY remained similar ($p=0.098$). In the control subgroup, at 5-months leptin concentration was reduced ($p=0.009$) in association with modest weight loss but concentrations of other hormones were not different from baseline. Plots displaying subgroup data are contained within the online appendix.

Predictors of weight regain

Baseline and within-trial hormone predictors of weight change are summarised in Table 2. There was a 2.3% (95% CI: 0.4, 4.1; $p=0.019$) increase in body-weight between 12 and 24-months for every 1 ng/ml increase in ghrelin between baseline and 12-months. There was no significant interaction effect between change in number of anti-diabetes medications and change in ghrelin concentrations in either treatment arm (data not shown). For every 1 ng/ml increase in leptin, body-weight increased by 0.5% (95% CI: 0.140, 0.835; $p=0.007$). Changes in concentration of GLP-1 and PYY between baseline and 12-months were not significant predictors of weight regain. Weight regain at 24-months was increased by 1.1% (95% CI: 0.2, 2.0; $p=0.023$) body weight for every 1 ng/ml increase in ghrelin at 12-months. No other predictors at 12-months were identified. In a subgroup of participants (n=56 intervention, n=22 controls) for whom blood samples were available for at 5-months, changes in appetite hormones between baseline and 5-months were not predictive of weight change at 12 or 24-months.

Discussion

Characterising the potential role of appetite hormones in the weight-reduced state is an important research objective given that they represent potential targets for anti-obesity

245 treatments (23). After diet-induced weight-loss, appetite hormones change in a direction that
246 seems to favour increased hunger and reduced satiety, but in the absence of evidence
247 correlating these changes with weight regain, the significance of these changes has remained
248 unclear. In this large cluster-randomised trial, the increase in fasting ghrelin that was
249 observed alongside weight change between baseline and 12-months was a predictor of weight
250 regain between 12 and 24 months, lending some support to the widely held view that
251 hormonal adaptations oppose long-term WLM. Concentration of ghrelin at 12-months also
252 predicted subsequent weight regain. Although effect sizes were modest and explain only a
253 small proportion of weight regain, attenuating the sustained rise in ghrelin in response to
254 weight-loss may have therapeutic benefit, the extent to which is likely mediated by the wide
255 individual variability in hormonal responses to weight-loss.

256 Ghrelin is the only gut hormone known to increase food intake and correlates with subjective
257 hunger (24). Ghrelin stimulates food intake by activating neurons within the hypothalamic
258 arcuate nucleus which co-express agouti-related protein (AgRP) and neuropeptide-Y (NPY),
259 both of which are potent appetite stimulating peptides (23). In this study, fasting ghrelin
260 increased by >40% in the intervention group following a 10% body weight-loss at 12-months
261 and this increase was sustained to 24-months even in the context of weight regain. In the
262 control group modest weight-loss between baseline and 24-months led to ghrelin levels
263 matching those in the intervention group suggesting that ghrelin is highly responsive to even
264 small weight-losses. Although the use of anti-diabetes medications such as metformin have
265 been shown to increase fasting ghrelin concentrations (25), there was no evidence suggesting
266 that this had a significant effect, in particular in the control group where antidiabetes
267 medication prescribing increased modestly between baseline and 12 months. Ghrelin appears
268 more sensitive to weight change than satiety peptides, which were reduced in intervention
269 participants but increased in controls throughout. Although increases in fasting ghrelin and

subjective hunger after weight-loss have been reported by several research groups, significant correlations with weight regain have generally been lacking (13-16, 18, 26). It is possible that elevations in ghrelin reflect changes in adiposity and a normalisation of the ghrelin profile since it negatively correlates with body mass index in people with and without type 2 diabetes (27, 28), however in the context of the available evidence we believe it is more likely that changes represent a compensatory drive to regain weight. The effects of increasing circulating ghrelin have been established, with significant increases in appetite and food intake observed in healthy individuals with and without obesity (29), as well as people with cancer and loss of appetite (30). Therapeutic strategies aimed at neutralising or blocking ghrelin activity could be a useful target for obesity treatments (31) and promising preclinical trials show that inhibiting ghrelin O-acyltransferase, the enzyme responsible for acylating ghrelin (and therefore its hunger promoting effects), may have potential for reducing energy intake and body weight (32).

Despite important roles in maintaining energy homeostasis, we did not find evidence that changes in satiety hormones after weight-loss contribute to weight regain, and several explanations are possible. In the weight-reduced state the brain is more sensitive to hunger signals than satiety (10), and satiety results from the cumulative action of several appetite hormones (33) making GLP-1, PYY and leptin less likely to be singularly predictive. In addition, our analyses were restricted to the fasting period, though effects of satiety hormones are greatest following a meal. Although not the 'gold standard' in appetite research, fasting measurements may have value in predicting treatment outcomes (34) and evidence suggests fasting and postprandial appetite hormones are positively correlated, in particular for ghrelin (35). Despite the lack of evidence implicating GLP-1 and PYY in weight regain, clear differences between intervention and control participants were evident, consequent to weight-loss. GLP-1 was surprisingly increased in controls but significantly reduced at 12 and 24-

295 months in the intervention group. The reduction in PYY observed in intervention participants
296 at 12-months did not reach statistical significance, but like GLP-1, in the subgroup there is a
297 suggestion that changes would have been greater at 5-months (see online appendix). Fasting
298 GLP-1 (13, 36, 37) and PYY (13, 38, 39) concentrations are usually reduced after weight-
299 loss, though sometimes levels are unchanged (32). No effects were found to support the role
300 of fasting GLP-1 or PYY in predicting weight regain, in agreement with other studies
301 investigating fasting and postprandial response (16, 18). An unresolved question relates to the
302 relevance of appetite hormones in the weight-reduced state. The circulating blood levels of
303 hormones may not necessarily reflect the concentrations that reach brain neurons, or their
304 physiological activities (40). Some, such as leptin, require an active transport mechanism to
305 enter the brain, which may be subject to other influences. As a result, one should not rule out
306 the possibility that changes in satiety hormones may indeed have an effect on WLM, however
307 our results suggest that at best, only small effects carrying little clinical implication would be
308 identified in any future studies with larger numbers.

309 As expected, leptin reduced after weight-loss and increased with regain, reflecting changes in
310 adiposity (41). Although regressions were corrected for changes in body weight, a higher
311 increase in leptin between baseline and 12-months was a predictor of regain 12-24 months.
312 The reasons for this finding are unclear but could be related to changes in body composition,
313 though this cannot be verified and requires further investigation.

314 Weight regain is multi-factorial and the overarching message of this paper is not to diminish
315 the role played by other non-homeostatic factors, which may be of equal or even greater
316 importance. The ‘voluntary choice’ versus ‘biological determinism’ debate regarding food
317 intake is interesting from an academic perspective (42), but in practice, behaviour results
318 from interactions between biology, environment and psychosocial factors. Our findings may

have practical implications for WLM. Despite an apparent biological response opposing WLM, weight regain should not be viewed as inevitable (43), and behavioural interventions may benefit from drawing on specific strategies known to have benefit in modifying appetite. Carbohydrate appears to be the most effective macronutrient in suppressing ghrelin due to postprandial insulin and glucose release whereas fat has a weak effect (44), and higher protein meals are more satiating and help to attenuate postprandial rises in ghrelin (45), possibly mediated by increasing concentrations of satiety peptides (46). People consume food by weight or volume so counselling individuals to incorporate more low-energy dense foods (e.g. soups, vegetables, fruits, legumes) helps to increase meal size whilst reducing hunger and energy intake (47). Physical activity is ineffective as a stand-alone weight-loss intervention but is an important WLM strategy (48). This may be explained partly because exercise acutely suppresses ghrelin, and increases GLP-1 and PYY (49), actions which reduce appetite and energy intake and do not seem to stimulate compensatory eating above the energy expended, as is often believed (50).

There are several limitations to this study. We focussed on the 'central players' involved in appetite regulation but many other hormones (40, 51, 52) and pathways are involved in WLM, including changes in energy expenditure (53), and investigating these may have provided additional insights. This is an exploratory analysis, the study was not designed to investigate the relationship between WLM and appetite hormone changes. Studies specifically designed to investigate the relationship between appetite hormones and weight changes may provide more definitive answers, and would enable subjective appetite measurements to be collected, although their reliability has been questioned (54). Blood samples were not treated with a protease or DPP-IV inhibitor which may have been beneficial under ideal conditions, although there is evidence that addition of aprotinin or DPP-IV inhibitors is not critical to obtain robust measurements of total GLP-1 and total PYY

(55, 56). Biochemical measurements were undertaken using multiplex assays which may provide less accurate and precise measurements compared with traditional ELISA platforms though low inter and intra-assay variation was re-assuring and previous data has shown favourable comparison of MSD assay results with singleplex immunoassays (57). The DiRECT study was not designed specifically to evaluate the relationship between appetite hormone concentrations and weight change and thus blood samples were not treated with a protease or DPP-IV inhibitor. Although under ideal circumstances this may have been beneficial, there is evidence that addition of aprotinin or DPP-IV inhibitors is not critical to obtain robust measurements of total GLP-1 and total PYY (55, 56). It would have been beneficial to have had blood samples available for the full study cohort at 5-months but data available at this time-point for the subgroup are indicative of the changes that take place in response to weight-loss (interventions) and relative stability (controls). Finally, it was not possible to obtain postprandial samples and therefore analyses were restricted to fasting only measurements.

This study provides some further important evidence to the hypothesis that compensatory changes in appetite hormones contribute to weight regain following diet-induced weight-loss. The rise in ghrelin that was observed in response to weight-loss remained elevated over time and predicted weight regain during follow-up. Although effect sizes are modest, attenuating the rise in ghrelin during diet-induced weight loss may improve long-term WLM outcomes. With a large sample size, inclusion of a control group and follow-up over a 2-year period, results from this dataset build on previous studies and shed new light on the relevance of appetite hormone changes for WLM.

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Conflict of interest

GT reports funding of PhD fees and conference expenses from Cambridge Weight Plan. WSL reports conference expenses from Cambridge Weight Plan. ACB reports lecture fees from Novo Nordisk and Napp Pharmaceuticals. NB was previously employed by Counterweight Ltd and reports personal fees for freelance work and shareholdings from Counterweight Ltd and funding of PhD fees and conference attendance from Cambridge Weight Plan. LM was previously employed by Counterweight Ltd and reports research funding from Cambridge Weight Plan and consultancy fees from Counterweight Ltd. NS reports research grants and speaker's honoraria from Boehringer Ingelheim and speaker's honoraria from Amgen, AstraZeneca, Eli Lilly, Janssen, Napp Pharmaceuticals, Novo Nordisk, and Sanofi. RT reports educational lecture fees from Eli Lilly and Novartis and advisory board fees from Wilmington Healthcare. MEJL reports research grants and personal fees for lecturing and consultancy from Novo Nordisk and consultancy fees from Counterweight Ltd, Novartis, and Eli Lilly. All other authors declare no competing interests.

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Table 1: Baseline characteristics

	Intervention group (n=147)	Control group (n=106)	Intervention subgroup (n=56)	Control subgroup (n=22)
Sex:				
Male	82 (56%)	67 (63%)	31 (55%)	13 (59%)
Female	65 (44%)	39 (37%)	25 (45%)	9 (41%)
Age	52.9±7.5	54.6±7.4	53.1±7.4	54.2±7.8
Weight (kg)	101.1±16.8	99.0±14.8	101.6±17.2	95.9±10.3
BMI (kg/m^2)	35.1±4.6	34.2±4.2	35.4±4.7	33.5±3.5

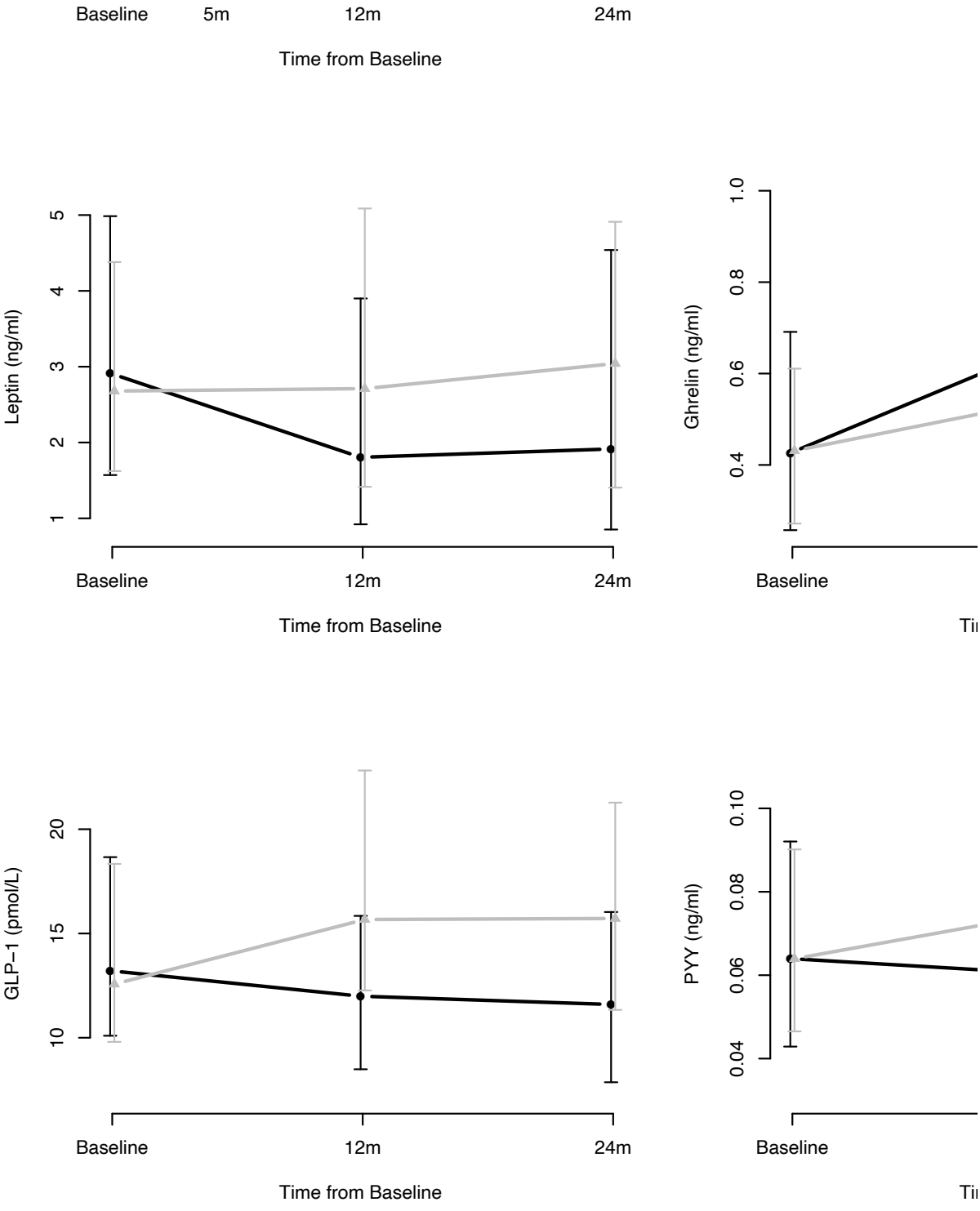

Figure 1. Baseline and within trial changes of body-weight and appetite-related hormones (leptin, ghrelin, GLP-1 and PYY) for intervention and control groups in DiRECT

Table 2: Baseline and within-trial predictors (leptin, ghrelin, GLP-1 and PYY) of weight change (%)

	Weight change effect (*β*, 95% CI) 5-12 months	**Weight change effect (*β*, 95% CI) 5-24 months**	**Weight change effect (*β*, 95% CI) 12-24 months**
Baseline leptin	0.150 (-0.218 , 0.541) ; p=0.446	0.047 (-0.382 , 0.458) ; p=0.830	-0.021 (-0.284 , 0.241) ; p=0.875
Δ Leptin 0-5 months*	0.329 (-0.386 , 1.044) ; p=0.394	0.662 (0.007 , 1.317) ; p=0.065	n/a
Δ Leptin 0-12 months	n/a	n/a	**0.488 (0.140 , 0.835) ; p=0.007**
12 month leptin	n/a	n/a	0.253 (-0.006 , 0.512) ; p=0.061
Baseline ghrelin	-0.264 (-1.861 , 1.507) ; p=0.756	0.778 (-0.947 , 2.664) ; p=0.401	0.985 (-0.262 , 2.232) ; p=0.129
Δ Ghrelin 0-5 months*	-2.534 (-7.407 , 2.338) ; p=0.336	0.749 (-4.048 , 5.546) ; p=0.772	n/a
Δ Ghrelin 0-12 months	n/a	n/a	**2.276 (0.417 , 4.134) ; p=0.019**
12 month ghrelin	n/a	n/a	**1.109 (0.178 , 2.040) ; p=0.023**
Baseline GLP-1	0.029 (-0.087 , 0.141) ; p=0.620	0.050 (-0.076 , 0.172) ; p=0.440	-0.033 (-0.105 , 0.040) ; p=0.389
Δ GLP-1 0-5 months*	-0.013 (-0.254 , 0.228) ; p=0.921	-0.088 (-0.321 , 0.145) ; p=0.482	n/a
Δ GLP-1 0-12 months	n/a	n/a	0.021 (-0.052 , 0.093) ; p=0.583
12 month GLP-1	n/a	n/a	-0.009 (-0.090 , 0.072) ; p=0.826
Baseline PYY	-9.737 (-34.007 , 15.697) ; p=0.450	7.019 (-19.974 , 35.096) ; p=0.624	4.538 (-11.368 , 20.445) ; p=0.583
Δ PYY 0-5 months*	-28.592 (-66.560 , 9.377) ; p=0.166	-12.927 (-48.904 , 23.050) ; p=0.506	n/a
Δ PYY 0-12 months	n/a	n/a	-9.666 (-27.234 , 7.903) ; p=0.291
12 month PYY	n/a	n/a	-2.157 (-17.862 , 13.547) ; p=0.792

GLP-1, glucagon like-peptide 1; PYY, peptide YY. *Hormone change between 0-5 months is for the subgroup only.

Results are presented as regression coefficients (*β)* and 95% CI for multivariate regression analyses of weight change between 5-12, 5-24 and 12-24 months after adjusting for age, sex, treatment group (intervention or control) and weight change between 0-5 months for the 5-12 and 5-24 month predictions, and weight change 0-12 months for 12-24 month predictions. Positive values indicate weight gain and negative values indicate weight loss for weight change effects. Statistically significant findings are shown in bold.

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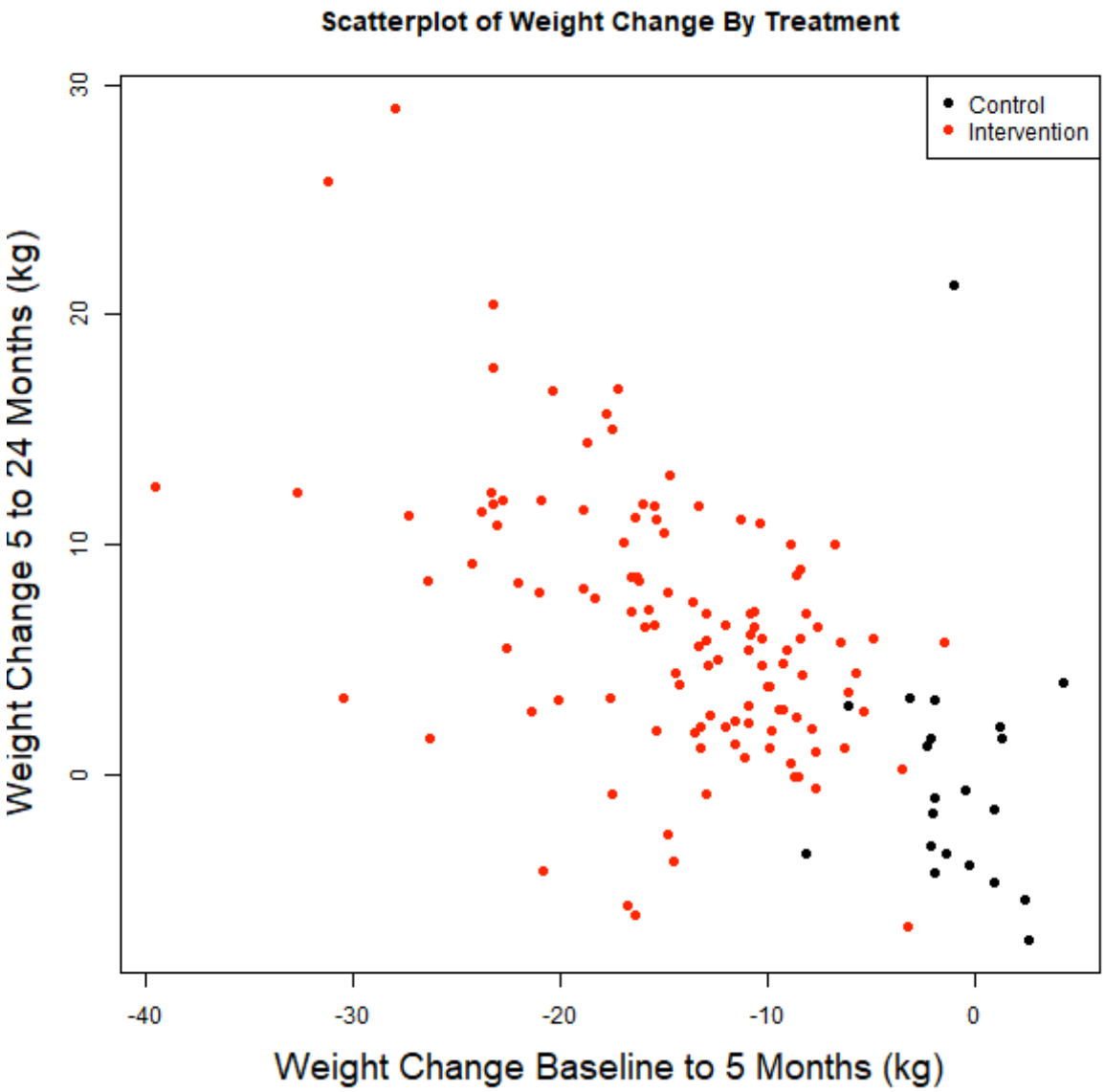

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609 **Figure 2:** Individual variability in weight-loss between baseline and 5-months and between 5 and 24-
610 months for the intervention group and a subgroup of control participants for whom weight change
611 data were available at 5-months.