Role of endogenous incretins in the regulation of postprandial lipoprotein metabolism

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ABSTRACT

Objective: Incretins are known to influence lipid metabolism in the intestine when administered as pharmacologic agents. The aggregate influence of endogenous incretins on chylomicron production and clearance is less clear, particularly in light of opposing effects of co-secreted hormones. Here we tested the hypothesis is that physiological levels of incretins may impact on production or clearances rates of chylomicrons and VLDL.

Design and Methods: A group of 22 overweight/obese men was studied to determine associations between plasma levels of glucagon-like peptides 1 and 2 (GLP-1, GLP-2), and glucose-dependent insulinotropic polypeptide (GIP) after a fat rich meal and the production and clearance rates of apoB48- and apoB100-containing triglyceride rich lipoproteins. Subjects were stratified by above- and below-median incretin response (area-under-curve).

Results: Stratification yielded sub-groups that differed about 2-fold in incretin response. There were no differences in apoB48 production rates in chylomicrons or VLDL fractions, nor in apoB100 or triglyceride kinetics in VLDL between men with above- versus below-median incretin responses. The men with above-median GLP-1 and GLP-2 responses exhibited higher postprandial plasma and chylomicron triglyceride levels, but this could not be related to altered kinetic parameters. No differences were found between incretin response sub-groups and particle clearance rates.

Conclusion: We found no evidence for a regulatory effect of endogenous incretins on contemporaneous chylomicron or VLDL metabolism following a standardised fat-rich meal. The actions of incretins at pharmacological doses may not be reflected at physiological levels of these hormones.

INTRODUCTION

Widespread recognition that elevated plasma triglyceride (TG) levels are linked with increased risk of atherosclerotic cardiovascular disease (ASCVD) has been accompanied by the challenge that we have yet to uncover the precise pathogenic mechanisms underpinning this relationship[1]. The levels of TG-rich lipoproteins (TRL) in the circulation are determined by their rates of production and clearance (1). While a great deal has been learned about the assembly and secretion of apoB100-containing VLDL in the liver (2), it is only recently that factors that regulate production of apoB48-containing particles in the intestine have been identified (1-3).

The hormones glucagon-like peptides 1 and 2 (GLP-1 and GLP-2 secreted from intestinal L-cells) and glucose-dependent insulinotropic polypeptide (GIP, secreted by enteroendocrine K-cells) are rapidly released into the circulation in response to meal consumption (3-7). These incretins have specific roles in the regulation of postprandial glucose handling and also in controlling lipid metabolism (3, 4, 7-14). Data from both animal models and clinical studies demonstrate that GLP-1 administered as a pharmacologic agent decreases chylomicron secretion (3, 15) while GLP-2 seems to enhance chylomicron production after fat ingestion (16, 17). The findings from these studies of individual incretins given at supra-physiological doses are informative, but their combined impact on postprandial lipid responses, especially at the levels seen normally in humans, is not yet clear. It is likely to be complex given that the two hormones have opposing actions on chylomicron levels, and are released simultaneously and in equimolar amounts. Clinical interest in the actions of incretins arises from the observation that GLP-1 agonists in humans diminish postprandial TG responses (although they have only modest effects on fasting TG levels) (3, 11, 13, 18-20). These actions of GLP-1 on postprandial lipemia and potentially on TRL remnants – the TG-depleted lipoprotein products of lipolysis that are thought to be particularly atherogenic (21, 22) – are considered to contribute to the cardioprotective benefits of GLP-1 agonists seen in ASCVD outcome trials (4, 23-25) and have prompted use of these agents in Type 2 diabetes subjects with atherosclerosis (26-28).

To date, there have been few human kinetic studies of incretin action on TRL metabolism. In a pioneering study, exenatide was reported to reduce acutely apoB48 production in healthy humans (15), and liraglutide appears to have a similar action in type 2 diabetic subjects (29-31). Also in type 2 diabetes, lixisenatide and liraglutide have been reported to accelerate removal of apoB48 - containing chylomicrons from the circulation (20, 29, 32). The present study builds on our earlier work on apoB48 kinetics (33, 34), and on the previous observation that the increases in GLP-1, GLP-2 and GIP levels following a standard fat-rich meal were all positively but weakly related to the degree of post-prandial lipemia (11). Our working hypothesis is that the incretins may impact on production or clearances rates of chylomicrons and VLDL to a greater degree than is evident from

the association with overall postprandial lipemia. A further possibility is that, given the opposing effects of GLP-1 and GLP-2, there may be no net influence of these hormones on chylomicron metabolism. Here we tested the hypothesis is that physiological levels of incretins may impact on production or clearances rates of chylomicrons and VLDL.

MATERIALS AND METHODS

Study subjects—We studied 22 healthy men with a wide range of plasma TG who had been recruited originally to a fructose supplementation intervention trial (Clinical Trials NCT01445730) (35). The 22 were selected from the larger group of 65 subjects if they had samples available for GLP-1, GLP-2, and GIP measurement, and were willing to undergo a postprandial lipoprotein kinetics investigation (36). The size of the present group was considered appropriate to reveal a 15-20% or greater difference in apoB production rate in those with above versus below median values for incretins. Subjects were overweight/obese men with waist >96 cm and body mass index (BMI) between 27 and 40 kg/m^2. Each subject underwent a physical examination and laboratory tests to exclude cardiovascular disease, uncontrolled hypertension, type 2 diabetes, and liver, renal, thyroid or haematological abnormalities. Exclusion criteria were regular daily alcohol consumption over 2 units (*i.e.,* 24 g ethanol) and use of medication or hormones affecting lipoprotein metabolism. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Helsinki and Uusimaa Hospital District. Each subject gave written informed consent prior to participation.

Study visits—Subjects underwent, on separate occasions (1) assessment of apoB48 and apoB100 kinetics using a protocol that included consumption of a standard fat-rich mixed meal, (2) an oral glucose tolerance test (OGTT), (3) determination of liver and intra-abdominal fat, and (4) measurement of heparin-releasable lipases (Supplementary Figure 1). Participants were instructed to avoid alcohol and strenuous exercise for 72 h before each study visit. Each subject kept a 3-day food diary to confirm that they followed their usual isocaloric diet during the entire study.

Oral glucose tolerance test—An OGTT was performed after an overnight fast. Subjects consumed 75 g of glucose and blood sampling was done before and 5, 10, 30, 60, 90, 120, 180 and 240 minutes after the glucose was consumed for determination of glucose, insulin, GLP-1, GLP-2 and GIP.

Determination of intra-abdominal fat depots—Proton magnetic resonance spectroscopy was performed with a 1.5 T whole-body device to quantify liver fat content (37). Magnetic resonance imaging was used to determine subcutaneous abdominal and intra-abdominal fat (38). Subjects were advised to fast for 4 h before the imaging.

Kinetic study— Kinetic studies were performed as previously described (29, 33, 34). After a 12-h overnight fast (at 8:00, 0-h time point), subjects received a bolus injection of 500 mg 2H5-glycerol (1,1,2,3,3-D5, Euriso-Top) and deuterated leucine (5,5,5-D3 Euriso-Top; 7 mg/kg body weight) to determine the kinetics of triglycerides, apoB100 and apoB48 in VLDL subfractions, and apoB48 in chylomicrons. At the 2-h time point, subjects were given a fat-rich mixed meal containing 927 kilocalories and consisting of bread, cheese, ham, boiled eggs, fresh red pepper, low-fat (1%) milk, orange juice and tea or coffee) served with a cocoa/fat emulsion containing 40 g olive oil (Amway, Firenze, Italy). This meal provided 63 g carbohydrate, 56 g fat and 40 g protein. Blood samples were taken at the 0-h time point and at frequent intervals until 10 h after tracer administration, when a dinner was served. The subjects returned the following morning to give blood at 24 h post tracer administration. Following the meal glucose, insulin, GLP-1, GLP-2 and GIP were determined at 30, 60, 120, 180, 240 and 360 minutes. Plasma lipid and lipoprotein levels were measured at 0, 30, 60, 120, 180, 240, 360 and 480 minutes. Up to the 10 h point, only water was consumed (*ad libitum)* and the subjects remained physically inactive.

Quantification of apoB48, tracer enrichment in apolipoproteins and triglycerides, multi-compartmental modelling and parameter estimation—Total plasma fasting and postprandial apoB48 levels were measured by immunoassay (Elisa kit, cat # 637-10641, Shibayagi Co. Ltd, Shibukawa, Gunma, Japan). The methodology for quantifying stable-isotope-labeled apoB48-peptides in plasma and lipoprotein fractions for enrichment studies, and the protocol for the kinetic investigation and the compartmental model structure have been described in detail previously (33, 34). Modelling and parameter estimation were performed using SAAM II (39). In the manuscript, we have employed the term 'production rate' to describe the appearance of labelled apoB48 in the bloodstream.

Lipoprotein isolation and biochemical analyses—Lipoprotein fractions [chylomicrons (S_f>400), large $VLDL_1$ particles (S_f60–400) and smaller $VLDL_2$ particles (S_f20–60)] were isolated by density gradient ultracentrifugation as described (40). TG and cholesterol concentrations were analysed in total plasma and lipoprotein fractions by automated enzymatic methods using the Cobas Mira S analyser (Hoffman-La Roche, Basel, Switzerland). GIP, GLP-1 and GLP-2 plasma concentrations were measured after ethanol extraction (70% vol/vol, final concentration) with immunological assays, as described (11). GIP and GLP-1 were measured using C-terminally directed assays, which detect both the intact peptide and the primary (N-terminally truncated) metabolite (41). The antiserum for GLP-2 is directed against the N-terminus and therefore measures only fully processed GLP-2 of intestinal origin (42). Remnant-lipoprotein cholesterol (RLP-C) was analysed using automated direct assays (Denka Seiken, Tokyo, Japan). Plasma levels of apoC-III were measured by

ELISA. All other biochemical assays used standard laboratory methods.

Statistical analyses and calculations—All statistical analysis was performed using R (version 4.0.2). Correlation coefficients were calculated by the Spearman method. P-values for the correlation coefficients were calculated using the function cor.test using R. Between-group p-values were calculated using the Mann-Whitney U-test. Adjustment for multiple testing was done using the false discovery rate (FDR) method. P-values in Figure 1 were calculated using repeated measures ANOVA. Regression models with total and chylomicron apoB48 production rates as the dependent variable were constructed to test for the combined explanatory potential of incretin responses to the fat meal. We based the power calculation on previous experimental data were apoB48 production was measured in an unrelated cohort. A two-sample t-test was used to determine power and number of subjects per sample using the mean and standard deviation from the subjects studied in earlier studies (29, 33, 43).

RESULTS

The subjects of the present kinetic study were 22 overweight/obese male volunteers who were a representative subset (**Supplementary Table 1**) of the cohort of 65 men that participated in an investigation of incretins and postprandial lipemia as reported previously (11). The potential association of incretin levels after the meal with chylomicron- and VLDL- production and clearance rates was explored in two ways. First, we divided the group into two on the basis of the incretin area-under-the-curve (AUC) response to the test meal being above or below the median value. This division was constructed for GLP-1, GLP-2 and GIP AUCs and the GLP-1 to GLP-2 ratio. The baseline characteristics of these sub-groups are given in **Supplementary Tables 2–5**. Second, we examined in the whole group correlations between GLP-1, GLP-2 and GIP AUCs and apoB48 production and clearance rates.

When subjects were divided into sub-groups based on the AUC of each incretin (GLP-1, GLP-2 and GIP) and the GLP-1/GLP-2 ratio (**Figure 1**), The postprandial response of plasma apoB48 did not differ significantly, in the comparisons of higher versus lower AUC (**Figure 1 D, I, N and S).** Likewise, chylomicron apoB48 did not differ significantly between higher and lower excursions in GLP-1 AUC, GLP-2 AUC or GLP-1/GLP-2 AUC groups (**Figure 1 E, J and T**). For chylomicron-TG there was a significantly higher excursion in the higher GLP-1 AUC sub-group (P=0.018) and a trend in the higher GLP-2 AUC sub-group (P=0.09) **(Figure 1 C and H).** In terms of total plasma TG, there was a tendency towards a higher excursion for the higher GLP-1 AUC group (P=0.094) (**Figure 1 B**) but not for the other stratifications (GLP-2 AUC (P=0.12) and GIP AUC (P=0.64) and GLP-1/GLP-2 AUC (P = 0.79) (**Figure 1 G, L and Q**). Significant differences in chylomicron- and

plasma TG and chylomicron-apoB48 were observed for GLP-1 and GLP-2 AUC when the same analysis was applied to the original cohort of 65 men (**Supplementary Figure 2**). It is likely, therefore, that the differences observed for GLP-1 and GLP-2 AUCs in the 22 subjects who underwent kinetic investigation were representative of the picture in the whole cohort. Examination of the sub-groups divided by GLP-1/GLP-2 AUC ratio revealed almost identical responses postprandially in all parameters measured despite the lower sub-group having a ratio 50% less than in the higher sub-group (**Figure 1 P-T**).

There were no discernible differences between sub-groups of subjects with higher versus lower GLP-1 AUC in the production or clearance rates for apoB100-containing VLDL1 or VLDL2 (**Table 1**). Likewise, triglyceride metabolism in VLDL1 and VLDL2 was not different between the sub-groups. Total apoB48 production was similar in the sub-groups as was basal or postprandial production of apoB48 into either the chylomicron, VLDL1 or the VLDL2 fractions (**Table 1**). A nominally significant difference was seen in the fractional catabolic rate of apoB48 and its triglyceride content in the chylomicron fraction (P=0.016 and P=0.01 respectively), both being higher in the sub-group with lower GLP-1 AUC. These observations may be indicative of an overall difference in clearance rates based on GLP-1 AUC. However, it should be noted that after adjusting for multiple testing, these findings were no longer significant (**Table 1**). The total clearance rate of apoB48 did not differ between the groups (P=0.56). Stratification according to GLP-2 and GIP AUC generated sub-groups that again showed no significant differences in the metabolic parameters for apoB100- and apoB48-containing lipoproteins or VLDL triglyceride metabolism (**Supplementary Tables 6** and **7**).

Exploration of the associations between the incretin AUCs and the production rate of apoB48 (**Figure 2**) revealed no significant correlation between GLP-1 AUC and total apoB48 production rate (r=0.053, P=0.82), nor between GLP-2 AUC and total apoB48 production rate (r=0.11, P=0.64) or between GIP AUC and total apoB48 production rate (r=0.031, P=0.89). We further investigated whether any of the components of total apoB48 production rate (secretion of apoB48 into either CM, VLDL1 or VLDL2) correlated with the incretin responses, and found no evidence for this (**Supplementary Figure 3**). Similarly, no significant correlations were observed between the chylomicron-TG fractional clearance rate and incretin AUCs (**Supplementary Figure 4**). In regression models, none of the incretin AUCs were predictive of the rates total apoB48 and chylomicron apoB48 (**Supplementary Tables 8** and **9**).

DISCUSSION

This investigation explored the relationship between endogenous incretins secreted after a fat meal and the production and clearance rates of apoB48-containing lipoproteins produced by the intestine, and apoB100-containing lipoproteins released by the liver. No evidence was seen of an effect of

endogenous GLP-1, GLP-2 or GIP on the rates of apoB48 secretion into the chylomicron or VLDL density ranges either when subjects were divided into those with a higher or lower incretin AUC (to maximise the possibility of revealing a relationship) or in correlation analyses. Likewise, as expected, there was no association of incretin AUC with apoB100 or triglyceride kinetics in VLDL1 or VLDL2. There were nominally significant higher clearance rates for chylomicron-TG and chylomicron-apoB48 in the subgroup with lower than median GLP-1 AUC, which may be meaningful in light of the reduced postprandial chylomicron-TG and plasma TG responses to the standard fat meal in these subjects (as seen in the 22 subjects undergoing kinetic investigation and in the whole cohort of 65 men) (11). The context of these findings is our prior observation that there were modest but significant positive associations of the postprandial response in plasma TG, chylomicron-TG, and chylomicron apoB48 levels with the AUC for GLP-1, GLP-2 and GIP (11). We hypothesised that the weak, aggregate relationships of endogenously produced incretins with the levels of lipid and apolipoproteins after a fat meal may reflect much stronger, possibly competing (e.g. GLP-1 vs GLP-2) actions of these hormones on the underlying rates of assembly and secretion, or of catabolism, of intestinally derived lipoproteins. However, we found no evidence to support this contention, and concluded that the substantial and sustained excursions in physiological levels of GLP-1, GLP-2 and GIP after the fat meal did not impact on contemporaneous assembly and secretion of apoB48-containing chylomicrons and VLDL-sized particles by the intestine.

The findings of the present study are in contrast to the recognised actions of incretins in animal models and in human studies using pharmacological doses of GLP-1 receptor agonists that mimic the effects of the incretin. All three incretins (GLP-1, GLP-2 and GIP) are thought to participate in the regulation of lipid homeostasis in humans based on a range of in vivo observations (6, 10, 13, 19, 44-46). A number of studies indicate that GLP-1 – more precisely GLP-1 receptor agonists – can act to regulate intestinal lipid metabolism leading to a reduction in triglyceride excursion after meals in healthy and type 2 diabetic subjects. The mechanisms underlying the action of GLP-1 on chylomicron production are complex and may be multiple, including effects on GI tract motility and gastric emptying (18, 30, 47-50). In contrast, GLP-2 appears to increase intestinal lipid secretion after meals in addition to its action on nutrient absorption and intestinal growth (16, 45). GIP is considered to modulate lipid homeostasis indirectly by increasing intestinal and adipose tissue blood flow and triglyceride uptake after meals thus favouring the storage of lipids for future needs (9, 46, 51-53). The difficulty in translating these observations to a more normal physiological setting is that the endogenous levels of incretins released after a fat meal are orders of magnitude lower than the pharmacological doses used in clinical trials (54, 55). Also, the simultaneous and equimolar release

of hormones with competing actions – GLP-1 and GLP-2 – will have a net action that is hard to predict.

In our study, GLP-1 plasma levels rose immediately on consumption of the test meal and remained 2- to 3-fold higher than fasting levels over the next 4 to 8 hours. It should be noted that circulating levels of incretins are likely to be lower than levels experienced by the intestine where they have a paracrine function (7, 23, 46). Chylomicron secretion from the intestine occurs throughout the period of alimentary lipemia, and it is predicted that if endogenous GLP-1 had an inhibitory action on apoB48 synthesis, as we observed with chronic administration of a GLP-1 agonist using the same metabolic protocol (29), then we should have seen a decrease in the production rate of the apoprotein and a diminished degree of alimentary lipemia in the higher GLP-1 sub-group. However, if anything, we saw the opposite effect with higher post prandial response in the above-median sub-group (both for the 22 subjects in the kinetic investigation and the whole cohort of 65 men). When the group was divided by GLP-2 AUC, those with the higher AUC had a greater increase in chylomicron TG and plasma TG than those with a lower AUC (a trend in the 22 men but significant in the whole group). This finding may be explained by the anticipated action of GLP-2 as a promoter of chylomicron secretion (10, 56). It should be noted that both in response to an OGTT and to the fat meal, the plasma levels of GLP-1 and GLP-2 were strongly and positively correlated with each other (and GIP) as expected (11), and the subjects of the present study when stratified by GLP-1 also had a higher GLP-2 AUC. The response curves relating TG excursion to GLP-1 and GLP-2 AUCs are potentially explained by GLP-2 having a dominant effect on chylomicron release. Interestingly, Stahel et al (56) reported that GLP-2 given intraperitonially 5 hrs after a high fat lipid bolus (Intralipid) increased lymph flow and promoted both TG and apoB48 output from intestine in rats. Recent studies further suggest that GLP-1 may influence intestinal lymph flow and output of triglycerides (57). Alternatively, other factors may have both increased the postprandial lipemic response to a fat meal and increased secretion of GLP-1 and GLP-2.

To date, the impact of GIP on lipid homeostasis has been little studied. The effects of the hormone on lipid homeostasis in the postprandial state appears to be explained by increases in blood flow in adipose tissue that result in greater lipid uptake, partly explained by the stimulatory effect of GIP on adipose tissue lipoprotein lipase activity (46, 51, 58, 59). Earlier studies demonstrated that the triglyceride response after a mixed meal or Intralipid load were similar, irrespective of whether subjects received a continuous GIP infusion or saline infusion (60).

The main limitation of this study is, as is common in kinetic investigations, the relatively small sample size used to derive statistical relationships. Our interpretation of the effects of incretins on chylomicron/apoB48 metabolism is based on associations between the combined incretin responses

to the standardised fat meal and kinetic variables rather than intervention with a specific hormone. This approach reflects better the physiological setting but may not reveal important mechanistic actions.

In summary, in this study we explored the potential relationships between incretins released from the gut after a standardised fat meal and the kinetics of apoB48-containing chylomicrons and VLDL. Given the findings of previous investigations (including our own using the same metabolic protocol) (11, 29) that GLP-1 receptor agonists have a profound effect on apoB48 synthesis, we anticipated that there would be an association between higher physiological level of this hormone and diminished chylomicron production. However, none was evident and, if anything, higher levels of GLP-1 were linked to a higher TG response to the meal. Similarly, no relationships were seen between postprandial GLP-2 and GIP levels and TG or apoB kinetics. We conclude, within the limitations of our experimental approach, that up to 2-fold concentration differences in GLP-1, GLP-2 and GIP exerted no discernable regulatory action on postprandial intestinal lipid metabolism.

Declaration of interest

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TABLES

GLP-1 stratified	Lower half (mean ± SD)	Upper half (mean ± SD)	P-value	Adj. P-value
ApoB100 production				
ApoB100 VLDL tot prod (mg/d)	1060 ± 250	1040 ± 280	0.87	1
ApoB100 VLDL1 Prod (pools/d)	733 ± 250	726 ± 320	0.97	1
ApoB100 VLDL2 prod (mg/d)	723 ± 180	796 ± 270	0.67	1
ApoB100 VLDL2 dirProd (mg/d)	323 ± 59	317 ± 61	0.73	1
VLDL1-TG prod (mg/d)	32 ± 21	30.8 ± 16	1	1
VLDL2-TG dir prod (mg/d)	4.3 ± 1.1	4.0 ± 0.9	0.34	1
Total VLDL-TG prod (mg/d)	37 ± 21	34.9 ± 15	1	1
ApoB100 clearance				
ApoB100 VLDL1 FCR (pools/d)	11.9 ± 8.9	9.8 ± 5.4	0.75	1
ApoB100 VLDL1 FDC (pools/d)	5.5 ± 4.8	4.1 ± 4.1	0.61	1
ApoB100 VLDL1 FTR (pools/d)	6.5 ± 4.9	5.7 ± 2.7	0.85	1
ApoB100 VLDL2 FCR (pools/d)	4.7 ± 1.7	5.4 ± 1.4	0.16	1
VLDL1-TG FCR (pools/d)	20.6 ± 16	17.1 ± 11	0.65	1
VLDL1-TG FDC (pools /d)	17.1 ± 15	14.2 ± 11	0.7	1
VLDL1-TG FTR (pools /d)	3.4 ± 2.2	2.9 ± 1.6	0.56	1
VLDL2-TG FCR (pools /d)	10.8 ± 5	10.9 ± 3.7	0.58	1
VLDL2-TG prod (pools /d)	9.6 ± 2.5	10.5 ± 4.9	0.77	1
ApoB48 production				
Total apoB48 prod (mg/d)	422 ± 150	440 ± 230	1	1
Basal apoB48 prod (mg/d)	38 ± 26	40.9 ± 24	0.62	1
Basal VLDL1-apoB48 prod (mg/d)	15.6 ± 13	14.5 ± 11	0.79	1
Basal VLDL2-apoB48 prod (mg/d)	22.4 ± 18	26.4 ± 22	0.69	1
CM-apoB48 prod (mg/d)	256 ± 170	248 ± 210	0.55	1
Postprandial VLDL1-apoB48 prod (mg/d)	70.5 ± 49	82.8 ± 62	0.84	1
Postprandial VLDL2-apoB48 prod (mg/d)	57.3 ± 42	68.7 ± 67	0.95	1
Postprandial VLDL-apoB48 prod (mg/d)	128 ± 56	151 ± 97	0.9	1
Basal apoB48-TG prod (mg/d)	1.5 ± 1	1.5 ± 0.9	0.97	1
ApoB48 clearance				
ApoB48 total FCR (pools/d)	19 ± 8.9	15.8 ± 8.2	0.56	1
ApoB48 CM FCR (pools/d)	56.1 ± 32	31.7 ± 29	0.016	1
ApoB48 CM-TG FCR (pools/d)	67.1 ± 37	32.4 ± 19	0.01	0.28
ApoB48 VLDL1 FCR (pools/d)	27.3 ± 19	28.9 ± 22	0.95	1
ApoB48 VLDL2 FCR (pools/d)	49.9 ± 17	61.2 ± 47	0.9	1

Table 1. Kinetic parameters of apoB100- and apoB48 metabolism for the lower- and upper half with the lowest and highest GLP-1 AUC respectively. Data are shown as mean and standard deviations. Standardized mean differences between the groups are shown. P-values are calculated using the Mann-Whitney U-test. Adjusted p-values are calculated using the FDR-method.

FIGURES

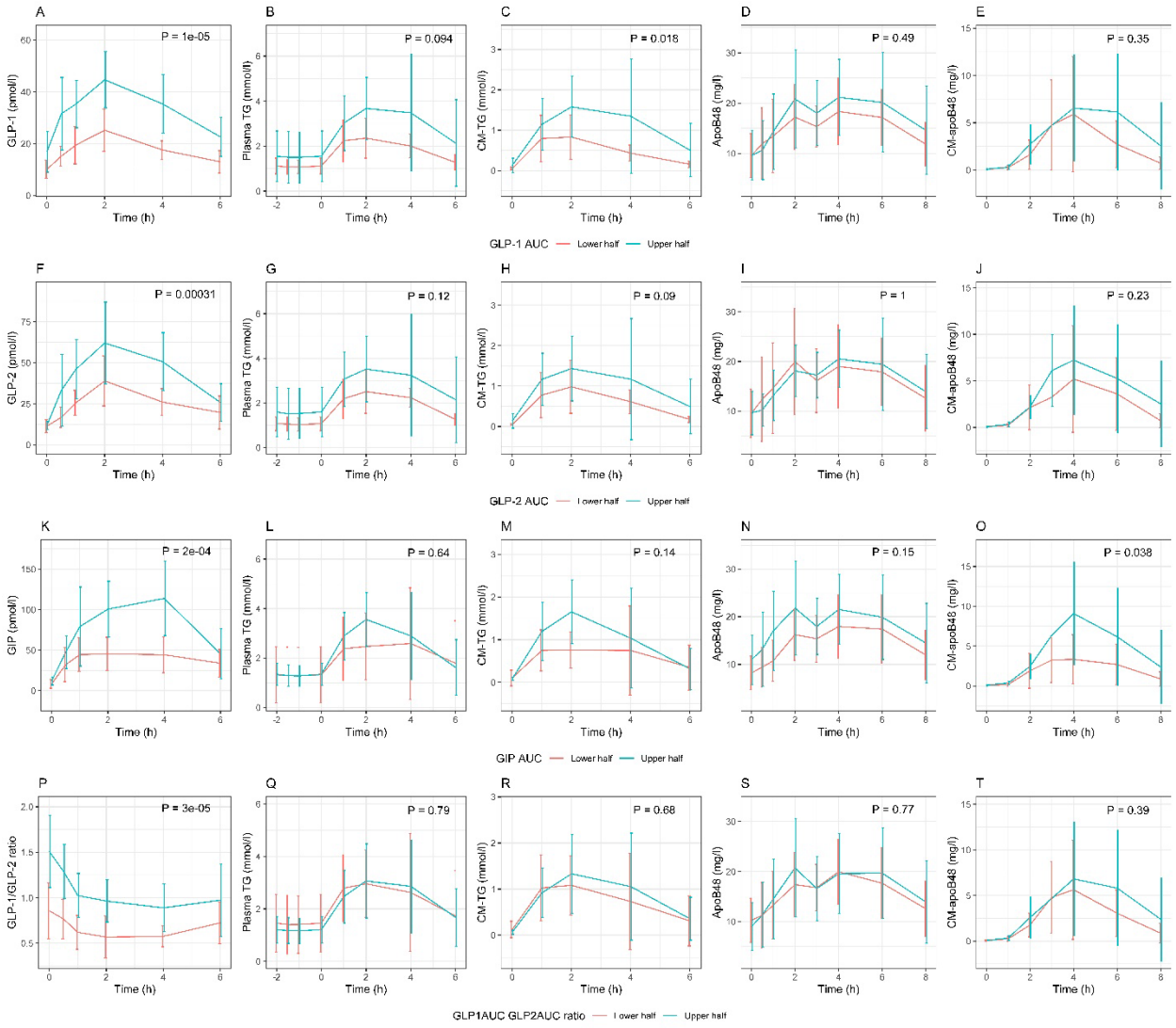
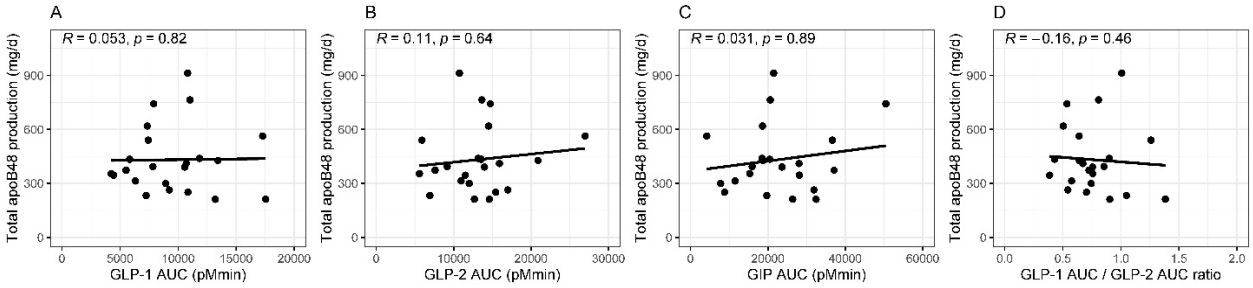


Figure 1. A-E) Postprandial excursions of GLP-1, CM-apoB48, Plasma apoB48, CM-TG and plasma TG are shown for the subjects with the lowest (red) and highest (green) GLP-1 AUC measure. Row 2, 3, and 4 show the same variables in column 2-5 but with differing stratifications; GLP-2 AUC, GIP AUC and GLP-1/GLP-2 AUC ratio respectively. Standard deviations are shown as error bars and p-values have been calculated using repeated measures ANOVA.

503 

504 **Figure 2.** Correlations between GLP-1 AUC, GLP-2 AUC, GIP AUC, GLP-1 AUC / GLP-2
505 AUC ratio and total apoB48 production rate. R-values refer to spearman correlation coefficients.

506