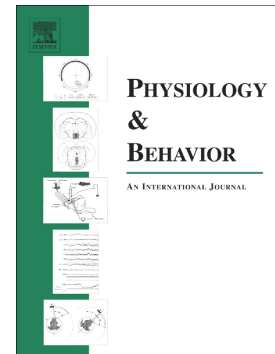


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Daily supplementation of dietary protein improves the metabolic effects of GLP-1-based pharmacotherapy in lean and obese rats

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Running Head: Dietary protein enhances sitagliptin's metabolic effects

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Abstract

Glucagon-like peptide-1 (GLP-1) is an incretin hormone released from intestinal L-cells in response to food entering into the gastrointestinal tract. GLP-1-based pharmaceuticals improve blood glucose regulation and reduce feeding. Specific macronutrients, when ingested, may trigger GLP-1 secretion and enhance the effects of systemic sitagliptin, a pharmacological inhibitor of DPP-IV (an enzyme that rapidly degrades GLP-1). In particular, macronutrient constituents found in dairy foods may act as potent secretagogues for GLP-1, and acute preclinical trials show that ingestion of dairy protein may represent a promising adjunct behavioral therapy in combination with sitagliptin. To test this hypothesis further, chow-maintained or high-fat diet (HFD)-induced obese rats received daily IP injections of sitagliptin (6mg/kg) or saline in combination with a twice-daily 8ml oral gavage of milk protein concentrate (MPC; 80/20% casein/whey; 0.5kcal/ml), soy protein (non-dairy control; 0.5kcal/ml) or 0.9% NaCl for two months. Food intake and body weight were recorded every 24-48h; blood glucose regulation was examined at baseline and at 3 and 6.5 weeks via a 2hr oral glucose tolerance test (OGTT; 25% glucose; 2g/kg). MPC and soy protein significantly suppressed cumulative caloric intake in HFD but not chow-maintained rats. AUC analyses for OGTT show suppression in glycemia by sitagliptin with MPC or soy in chow- and HFD-maintained rats, suggesting that chronic ingestion of dairy or soy proteins may augment endogenous GLP-1 signaling and the glycemic- and food intake-suppressive effects of DPP-IV inhibition.

Introduction

There is an imperative need to find safe, effective, and economically achievable therapies for the treatment of type II diabetes mellitus (T2DM) and obesity given the increasing health and economic burdens associated with these diseases [1, 2]. Despite our ever-growing understanding of the environmental, anatomical, physiological, molecular, neuronal, and behavioral mechanisms that contribute to obesity/T2DM, it is clear that we currently have limited therapeutic options for either disease. Thus, leading theories on how to treat both obesity and T2DM in the vast majority of the population suggest that treatments involving a combination of behavioral lifestyle modification (e.g. altering diet) and pharmacotherapy would be most effective [3-6]. Given the clear association between T2DM and obesity, and the overlapping contribution of the glucagon-like peptide-1 (GLP-1) system in control of blood glucose and food intake regulation, it seems logical to explore whether behavioral modifications that include greater ingestion of specific food groups would enhance the blood glucose-, food intake-, and/or body weight-lowering effects of GLP-1 pharmacotherapies. Epidemiological data combined with accumulating basic science reports from cell lines and animal models support the perspective that regular consumption of dairy foods may have a beneficial role in body weight and glycemic management, as well as the prevention of metabolic syndrome [7-13], potentially through mechanisms involving enhanced GLP-1 signaling [14, 15].

GLP-1 is released from intestinal L-cells in response to nutrient entry into the gastrointestinal tract [14, 16, 17], but is rapidly degraded by the enzyme DPP-IV. Previous research suggests that the intrainestinal presence of specific bioactive

components, whole proteins, and select amino acids found within complete dairy protein [a.k.a. milk protein concentrate (MPC); ~80% casein / ~20% whey] is linked with release of insulin and gut peptides, including GLP-1 [18, 19], as well as suppression of food intake [12, 20-25]. Thus, foods such as dairy products can act as potent GLP-1 secretagogues when ingested, suggesting that it may be possible to improve blood glucose regulation and reduce food intake by combining select foods with an injection of a DPP-IV inhibitor, such as sitagliptin. Indeed, as sitagliptin and other DPP-IV inhibitors are currently FDA-approved for the treatment of T2DM (see [26] for review), increasing intake of a particular type of food (e.g., MPC) may represent a straightforward way to enhance the efficacy of currently used pharmacological diabetes treatments.

The hypothesis that the presence of MPC within the small intestine can augment endogenous GLP-1 signaling is supported by clinical [24, 25, 27-30] and basic science [31-33] reports showing that ingestion or intrainestinal infusion of dairy proteins, oligopeptides, and isolated amino acids can elevate post-prandial plasma GLP-1 and other intestinally-derived gut peptide (e.g. cholecystokinin) concentrations. More importantly, we have previously demonstrated that the acute combination of DPP-IV inhibition with an intraduodenal infusion of MPC resulted in a significant enhancement in the suppression of acute (30min) food intake and blood glucose concentrations in lean non-diabetic rats [33]. What remains to be determined is whether these effects are recapitulated with long-term daily ingestion of MPC combined with DPP-IV inhibition in lean/euglycemic and obese/hyperglycemic rats. Therefore, we tested whether chronic administration of MPC combined with sitagliptin improves measures of glycemic and energy balance control. Further, our acute studies previously showed that there are

unique metabolic effects of dairy-derived proteins [33]. To assess whether these MPC-specific effects also exist over the longer term, or if any higher-protein diet might deliver the same enhanced beneficial responses when combined with DPP-IV inhibition, additional lean and obese rats ingesting daily isocaloric soy protein are examined.

Methods

Animals. Adult male Sprague Dawley rats were obtained from Harlan (ordered at 355-370g). Animals were singly housed in hanging wire mesh cages in a temperature- and humidity-controlled environment on a reverse light-dark cycle. Food and water were available *ad libitum* except as noted below. All procedures were approved by the University of Pennsylvania Institutional Animal Care and Use Committee and conform to the NIH Guide for the Care and Use of Laboratory Animals.

Protein supplements and drug. Milk protein concentrate (MPC, a.k.a. milk protein isolate; 85%, Idaho Milk Products) and soy protein shake (natural flavor, Genisoy) were emulsified in deionized water with 2% Tween 80 such that the final solutions each provided 0.5 kcal/ml. Saline (0.9%) with 2% Tween 80 was used as a non-caloric control solution. All solutions were brought to a pH of 7.0-7.4 prior to gavage. Sitagliptin (Biovision) was dissolved in sterile 0.9% NaCl for intraperitoneal (IP) injections.

Experimental design. Separate groups of rats were maintained on chow (Purina 5001) or a 60% high-fat diet (HFD; Research Diets) for this study. Importantly, the HFD-fed

rats were maintained on the diet for approximately one month prior to testing to generate diet-induced obesity. Rats were also habituated to IP injection and oral gavage procedures for one week before testing. Rats within each dietary condition were assigned to receive once-daily IP injection of the DPP-IV inhibitor sitagliptin (6 mg/kg) or vehicle (0.9% NaCl, 1 ml/kg) in combination with twice-daily oral gavage of either MPC, soy protein, or saline (8 ml per gavage, 16 ml total per day). This was a total of 12 conditions (2 diet x 2 drug x 3 protein; n=10/condition). Although sitagliptin is usually administered orally in humans, IP injection was used for these studies rather than administering the sitagliptin in the rats' food or water. This was because rats in different diet/drug/protein conditions would likely consume different amounts of food (and, as a consequence, would also drink different amounts of water) over the course of testing, leading to variability in dosing if drug was given via food or drinking water.

Each day, the IP injection and first oral gavage were administered approximately one hour after the onset of the dark phase, and the second oral gavage was given approximately 8 hours later (e.g., 9 hours after dark onset). Treatments lasted for 9 weeks. Rats were run over 2 waves, with identical timing and methods between waves and all groups represented in each wave. To evaluate changes in energy balance over the ~2 month study, body weight was recorded daily for all rats at the beginning of the dark phase. Food intake was measured every 48h near the beginning of the dark phase; crumb spillage was collected and accounted for in these measurements. Cumulative energy intake and body weight gain over the 9 weeks of treatment were the measures of interest. Glycemic control was evaluated via oral glucose tolerance test (OGTT) administered after 3 weeks and again after 6.5 weeks of treatment. Within each

wave, each OGTT was run on two consecutive days, with half the rats run on one day and the remaining animals the next day. An equal distribution of treatment conditions was maintained on each OGTT experimental day.

On days when OGTT was conducted, food was removed from the cage at the onset of the dark phase, and OGTT started approximately 5h later. Each rat still received its assigned daily IP injection of either saline or sitagliptin and protein/vehicle gavage 1h after dark onset. Food remained unavailable until the OGTT was complete. Water was available before and after the OGTT, but was removed during the test. For each rat, small blood samples were taken from the tail tip to evaluate baseline blood glucose (BG) levels using a standard glucometer (AccuCheck). The rat then received an oral gavage of glucose (2 g/kg, time = 0min) and subsequent blood glucose readings were taken at 20, 40, 60, and 120min after gavage.

Data analysis. For energy balance data, only rats with complete data sets were included in statistical analyses. Fifteen rats were removed from analyses due to subject attrition or technical errors (e.g. errors in measurements). When technical errors in food measurements occurred, the missing data were calculated from the average of the individual animal's readings before and after the error occurred. If more than 3 readings in a row had issues, the animal was eliminated from the study. If an animal received an incorrect drug or protein on a given day, the energy intake / body weight data for that day were removed and, if applicable, accounted for as described above. Rats with food intake / body weight data that were consistent statistical outliers (e.g., more than 2 SD above or below the mean) were excluded from analysis. Cumulative energy intake data

accounted for kilocalories from food, protein supplementation, and glucose during OGTTs. Energy intake from food only (e.g., without kcal from protein supplementation or glucose) and glucose only were also evaluated. For OGTT data, percent change in blood glucose was calculated based on glucose levels at time 0, and AUC data were calculated based on the percent change curves. Statistical outliers were also removed for each OGTT.

All statistical analyses were run using Statistica (StatSoft). A p-value less than 0.05 was considered to be statistically significant. For cumulative energy intake and cumulative body weight data, between-subjects ANOVAs were run, including factors of diet, drug, and protein. Baseline body weight data and blood glucose data were analyzed by one-way ANOVA with diet as the between-subjects factor. For each OGTT, percent change in blood glucose over time was analyzed by a single mixed-design ANOVA, accounting for the within-subjects factor of time and the between-subjects factors of diet, drug, and protein. AUC data were analyzed similarly, in a single ANOVA for each OGTT with factors of diet, drug, and protein. Although data for each OGTT were analyzed in a single large ANOVA, data are shown by diet for clarity. All data are shown as mean \pm SEM.

Results

Energy intake and body weight gain. The energy balance effects of once-daily sitagliptin (vehicle or 6 mg/kg sitagliptin, IP) and twice-daily protein supplements (MPC, soy, or saline) were evaluated in lean chow-fed rats and obese HFD-fed rats (body weight on treatment day 0: chow = 389.00 ± 1.54 g; HFD = 454.12 ± 2.78 g; $F_{1,103}=424.19$, $p<0.01$).

The cumulative energy intake of rats in each diet/drug/protein group, including kilocalories from food, protein supplementation, and glucose during oral glucose tolerance testing (OGTT), was monitored for the 9 weeks of treatment ($n=105$; $n=7-10$ per diet/drug/protein condition). As shown in Fig. 1A, HFD-maintained rats consumed significantly more energy than chow-fed animals (main effect of diet, $F_{1,93}=26.92$, $p<0.00001$). Furthermore, an interaction between diet and protein type was observed ($F_{2,93}=3.34$, $p<0.04$); posthoc analyses revealed that HFD-fed rats given twice-daily oral gavage of saline had higher energy intake compared to all other diet/protein combinations ($p<0.05$). Total energy intakes from maintenance diet alone (kcal from only chow or only HFD) and from glucose alone were also evaluated. Energy intake from food is shown in Fig. 1B; HFD-fed rats consumed more kcal from the maintenance diet than chow-fed animals (main effect of diet, $F_{1,93}=27.26$, $p<0.00001$). Protein supplementation impacted intake of maintenance diet (main effect of protein, $F_{2,93}=62.87$, $p<0.000001$); saline-supplemented rats consumed the most energy from their maintenance diet compared to soy- or MPC-supplemented rats ($p<0.05$), which is unsurprising given the extra kcal provided by protein supplementation. An interaction between diet and protein type was observed for kcal from maintenance diet ($F_{2,93}=3.24$, $p<0.05$). Posthoc tests showed that food intakes of the saline-treated rats were different from all soy- or MPC-supplemented groups ($p<0.05$), with HFD/saline-treated rats ingesting more energy from food than chow/saline-treated rats ($p<0.05$). HFD/soy-treated rats also consumed more energy from food than chow/MPC-treated animals ($p<0.05$). Finally, energy provided by glucose during OGTT was examined (Fig. 1C). HFD-maintained rats received more kcal of glucose than chow-maintained rats (main

effect of diet, $F_{1,93}=174.60$, $p<0.000001$). This is due to the fact that glucose was administered as a function of body weight, and though HFD-fed rats weighed more, it is important to note that energy from glucose represents only a very small percentage of total kcal.

Cumulative body weight gain was also measured for the duration of the experiment; these data are shown in Fig. 2A, with growth curves for each treatment group shown in Fig. 2B-C. For cumulative weight gain, only a significant main effect of diet was observed ($F_{1,93}=6.09$, $p<0.02$). Chow-fed rats displayed increased weight gain compared to HFD-fed rats. The differences in magnitude of weight gain between diet conditions may be due to the fact that the HFD-fed animals weighed more at the beginning of testing and gained less weight over time.

Glycemic control. Oral glucose tolerance tests were used to evaluate glycemic control at 3 weeks and 6.5 weeks into the drug/protein treatments. It is important to note that the HFD-fed rats were hyperglycemic relative to the chow-fed animals; after a 5h fast, baseline blood glucose levels at the time of the first OGTT were 104.91 ± 1.17 mg/dl in HFD-fed rats compared to 95.07 ± 1.06 mg/dl in chow-maintained animals ($F_{1,89}=38.75$, $p<0.000001$).

Data from the first OGTT are shown in Fig. 3 ($n=91$; 5-9 per diet/drug/protein condition). After 3 weeks of drug/protein treatments, a significant time x diet x drug x protein interaction was observed ($F_{6,237}=3.10$, $p<0.01$). Posthoc analyses revealed that in chow-fed rats, rats treated with sitagliptin/saline had a reduced change in blood glucose from 20-60min compared to vehicle/saline-treated rats; sitagliptin/MPC also

reduced the percent change in blood glucose at 40min, suggesting that these treatments improved glycemic control. The sitagliptin/saline group also had significantly lower values compared to the vehicle/soy-treated rats from 20-60min. In the HFD-fed rats, each sitagliptin-treated group had a reduction in blood glucose levels: sitagliptin/saline at 40 and 60 min, sitagliptin/soy at 20 and 60 min, and sitagliptin/MPC at 20 and 40 min. AUC data (Fig. 3B and 3D) support these general findings; sitagliptin reduced AUC (main effect of drug; $F_{1,79}=50.47$, $p<0.00001$). Furthermore, an interaction was found between drug x protein ($F_{2,79}=4.24$, $p<0.02$). Posthoc analyses showed that all sitagliptin-treated groups have a lower AUC than the vehicle/saline-treated rats; interestingly, MPC alone (vehicle/MPC) also produces lower AUC.

The second OGTT was conducted approximately 3.5 weeks later, after 6.5 total weeks of treatment (Fig. 4; $n=95$; 7-9 per diet/drug/protein condition). The percent change in blood glucose was again decreased in sitagliptin-treated animals (main effect of drug, $F_{1,83}=23.51$, $p<0.00001$). A significant interaction between time and drug ($F_{3,249}=13.90$, $p<0.00001$) showed that sitagliptin lowered the change in blood glucose from 20-60min after oral glucose gavage. Significant interactions between time x diet ($F_{3,249}=34.43$, $p<0.00001$; posthoc different at 20 and 120 min) and time x protein ($F_{6,249}=2.16$, $p<0.05$; posthoc revealed no differences between groups within each timepoint) were also observed, but the 4-way interaction did not reach statistical significance (time x diet x drug x protein, $F_{6,249}=1.44$, $p=0.20$). AUC data indicated that sitagliptin improved glycemic control after 2 months of treatment (main effect of drug, $F_{1,83}=18.96$, $p<0.0001$).

Discussion

DPP-IV inhibitors are an effective FDA-approved therapy for the treatment of T2DM (see [26] for review). Importantly, there is still a need for enhanced glycemic control in the vast majority of patients prescribed these GLP-1-based pharmacotherapies. Thus, identifying complementary dietary behaviors that can augment the metabolic portfolio of effects produced by chronic DPP-IV inhibition is of major clinical importance. Whole proteins and isolated amino acids from dairy elicit GLP-1 secretions *in vitro* [18, 34] and inhibit intestinal DPP-IV activity *in vivo* [35], supporting the hypothesis that a diet rich in protein may enhance the glycemic effects of sitagliptin. To this end, our previous analyses showed that intraduodenal infusion of MPC, but not soy protein, significantly enhances both the short-term food intake- and glycemic-suppressive effects of sitagliptin [33]. Here, we examined whether these effects could be recapitulated chronically with daily DPP-IV inhibition combined with daily protein supplementation in both lean and high fat diet-induced obese rats for ~2 months. Accounting for the calories derived from the protein supplementation, current findings reveal that daily supplementation of either MPC or soy protein significantly suppresses cumulative caloric intake in HFD but not chow-maintained rats. Further, sitagliptin by itself or in combination with MPC or soy in chow- and HFD-maintained rats resulted in suppression in glycemia. Collectively, current data suggest that chronic ingestion of dairy or soy proteins may augment endogenous GLP-1 signaling, suppress food intake, and potentially provide glycemic benefits.

While DPP-IV inhibitors are less potent in their glycemic-inhibitory effects compared to GLP-1R agonists, they offer humans a number of advantages. Namely,

T2DM patients prescribed DPP-IV inhibitors can conveniently consume the drug orally and have reduced incidence of adverse gastrointestinal effects (e.g. nausea/vomiting) [36, 37]. There is general consensus in the literature that chronic DPP-IV inhibition does not result in sustained suppression of food intake or body weight (see [16, 26] for review). However, a previous report by Reimer et al. [38] showed that chronic DPP-IV inhibition in mice produces sustained intake suppression in either standard rodent chow or high fat diet. While we did not observe a significant suppression of chow or HFD intake by sitagliptin in the current set of studies, a number of differences might explain the discrepancy with the aforementioned study. We chose to deliver sitagliptin treatments via an IP route of administration instead of dissolving the drug in the food or water, as was done by Reimer et al. [38], to avoid any potential dosing confounds that may occur with variable day-to-day ingestion of food and/or water. Additionally, we also note that we tested the effects of sitagliptin administration on rats, whereas the aforementioned study examined the effects of the DPP-IV inhibitor DPP728 on mice. Thus, future research is certainly warranted to examine whether varied dosing and/or specific DPP-IV inhibitors can be used with or without daily dietary protein ingestion to observe any meaningful reductions in food intake that may be clinically relevant.

An important limitation to the current findings is the absence of plasma GLP-1 and insulin measures. Sitagliptin would be expected to increase plasma GLP-1 levels, but the incretin effects of GLP-1 may have also altered insulin levels in the bloodstream. Furthermore, it is unclear whether the expected increase in GLP-1 produced by the chronic treatments was the result of a putative increase in GLP-1 secretions by L-cells, and/or a consequence of reduced bioactive DPP-IV levels. Sitagliptin clearly increases

plasma GLP-1 in Sprague Dawley rats [39], as well as in models such as the fatty Zucker rat [40, 41] and a streptozotocin-induced diabetic rat model [42]. Studies measuring the effects of dairy protein on GLP-1 levels *in vivo* are less common, but a few studies in humans indicate that milk [43] and whey protein [19] increase plasma GLP-1 levels. Additionally, *in vitro* reports have shown that when GLP-1-producing L-cells are exposed to specific amino acids (i.e. leucine and isoleucine), there is an increase in GLP-1 secretion [18, 34]. Collectively, these data suggest that GLP-1 was likely increased by chronic sitagliptin and/or MPC treatment in our animals. However, multiple oligopeptides can also act as endogenous inhibitors of DPP-IV itself [35, 44] and whey protein as a whole can reduce DPP-IV activity in the proximal small intestine, the predominant site of GLP-1 secretion in the intestine [35]. Thus, there is clear need for future mechanistic evaluations to examine the impact that daily protein supplementation has on incretin hormone levels in general circulation, portal vein circulation, lymph and intestinal brush-border (see [16, 26, 45, 46] for review on endogenous GLP-1 mode of signaling).

One interesting finding from the current studies is that rats maintained on either soy protein or MPC showed comparable improvements in total caloric intake and glycemia alone in HFD-maintained rats. Furthermore, protein supplementation largely influenced overall caloric intake with no significant effect of sitagliptin alone. The fact that overall energy intake was affected by protein supplementation only in HFD-maintained animals was intriguing and may be explained by differences in protein content of the maintenance diets. The rodent chow used in our studies is approximately 30% kcal from protein, whereas the HFD is approximately 20% kcal from protein. The

protein supplement may have more robust or at least more detectable effects on caloric intake in animals whose “everyday” diet contains a lower percent of kcal from protein, e.g. in the HFD-fed rats.

In contrast, sitagliptin was the major factor in improving glycemic control; by and large, the addition of either protein did not generate a robust enhancement of the effects of sitagliptin alone. It is possible that no further significant enhancement in glycemic control was generated by the combination of sitagliptin and MPC or soy protein due to a floor effect, particularly in the case of MPC, where MPC alone was able to suppress blood glucose in the first OGTT. Interestingly, a significant drug x protein interaction on AUC was observed during the first OGTT, but was no longer statistically significant by the time of the second OGTT. Comparing the AUC data from the two OGTTs, it appears that in the vehicle-treated HFD-fed rats, protein had a more robust suppressive effect on AUC during the first OGTT that is largely absent during the second OGTT. It may be that this difference contributed to the significant drug x protein interaction during the first OGTT, but perhaps due to habituation to the glycemic-suppressive effects of protein supplementation over time, this effect was blunted by the time of the second OGTT and the interaction was no longer significant. Future dose-response analyses of sitagliptin, as well as studies examining the effects of other protein and/or amino acid constituents with sitagliptin may help to further examine and optimize the combinatorial potential of combining dietary protein ingestion with DPP-IV inhibition.

Collectively, these preclinical studies offer compelling evidence to suggest that chronic maintenance on a twice-daily dietary supplementation of dairy- or soy-derived protein is sufficient to reduce total caloric intake in rats maintained on a high-fat diet.

Perhaps more interestingly, following one month of maintenance on a twice-daily protein supplementation, MPC, but not soy, was able to significantly suppress glycemia as measured by an OGTT. Sitagliptin, on its own or in combination with either MPC or soy, also led to a sustained suppression in blood glucose. Altogether, these experiments offer evidence to support the hypothesis that daily ingestion of a complex protein source may be beneficial as an adjunct behavioral therapy to combine with GLP-1-based pharmacotherapies for the treatment of T2DM. Future studies are warranted to investigate whether these effects are translatable to humans with metabolic syndrome or T2DM.

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Figure Legends

Figure 1. Total energy intake (A) in all treatment groups over the two months of treatment. For energy intake, a main effect of diet is observed; HFD-maintained rats consume more energy. Additionally, a diet x protein interaction was observed; HFD-fed rats treated with saline consumed more energy than any other diet/protein combination. Below, the portions of total energy intake that come from food (B) and glucose (C) are shown. Main effects of diet and protein were found for total food intake, with HFD-fed rats eating more food than chow-fed rats, and saline-supplemented rats consuming more energy from food than soy- or MPC-supplemented animals. A diet x protein interaction was also observed; saline-treated rats consumed more kcal from food, with rats in the HFD/saline conditions ingesting more kcal from food than the chow/saline rats. For glucose, only a main effect of diet was found; HFD-fed rats received more kcal from glucose than chow-fed rats. *, significant effect of diet ($p < 0.05$). In panels A and B, the letters above the bars represent group differences in the diet x protein interaction; bars with different letters are significantly different from each other ($p < 0.05$). Data are mean \pm SEM.

Figure 2. Cumulative body weight gain (A) in all treatment groups over the two months of treatment. A main effect of diet on body weight gain was observed; HFD-fed rats gained less weight than did chow-fed animals. Data are mean \pm SEM. The growth curves of chow-fed animals (B) and HFD-fed animals (C) are also presented; data reflect body weight means. *, significant effect of diet ($p < 0.05$).

Figure 3. Results of an oral glucose tolerance test after 3 weeks of drug/protein treatment. In chow-fed rats (A), reductions in the percent change in blood glucose levels were observed in rats treated with sitagliptin/saline (sit/sal) from 20-60min and with sit/MPC at 40min after oral glucose gavage. The sit/sal group was also significantly different from the vehicle (veh)/soy group from 20-60min. In HFD-fed rats (C), the percent change in blood glucose was suppressed by sit/sal at 40-60min, sit/soy at 20 and 60min, and sit/MPC at 20-40min. When the data are analyzed as area under the curve (B, chow-fed; D, HFD-fed), a significant drug x protein interaction reveals that all sitagliptin-treated groups have significantly lower AUC values compared to veh/sal, but interestingly, MPC alone (veh/MPC) also produces a significant decrease in AUC. *, significantly different from veh/sal ($p < 0.05$); #, significantly different from sit/sal ($p < 0.05$). The key in A also applies to panel C. Data are mean \pm SEM.

Figure 4. Results of an oral glucose tolerance test after 6.5 weeks of drug/protein treatment. The percent change in blood glucose over time is shown for chow-fed rats in panel A and in high-fat diet fed rats in panel C. Sitagliptin significantly reduced the change in blood glucose at 20, 40, and 60 min after oral glucose gavage (time x drug interaction). A significant time x diet interaction was also observed at 20 and 120min. When the data are analyzed as area under the curve (B, chow-fed; D, HFD-fed), a main effect of drug is observed such that sitagliptin significantly suppresses AUC values. In A and C, * represents a significant time x drug interaction ($p < 0.05$), # indicates a significant time x diet interaction ($p < 0.05$). In panels B and D, * represents a significant

main effect of drug ($p < 0.05$). The key in A also applies to panel C. Data are mean \pm SEM.

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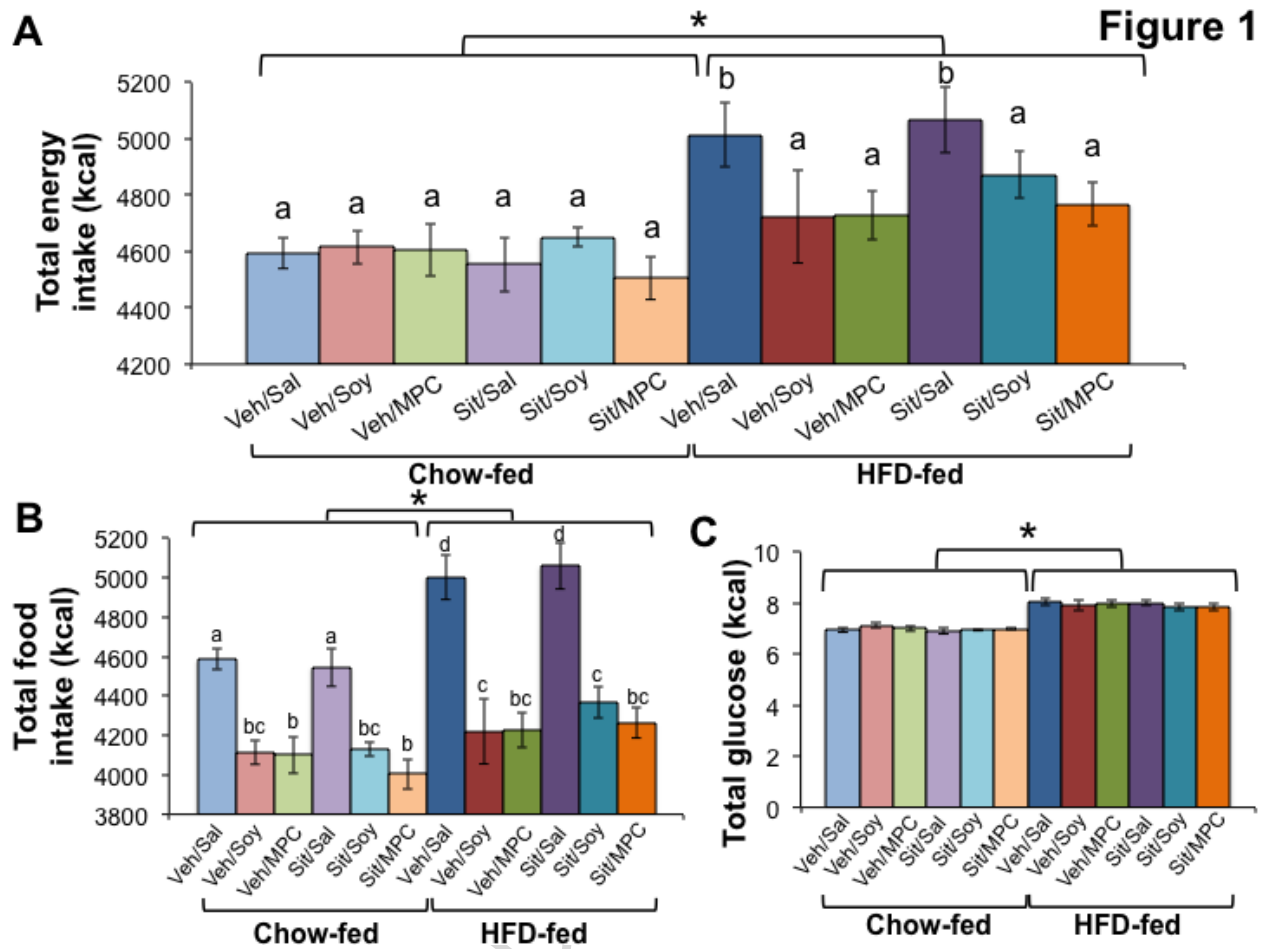


Figure 1

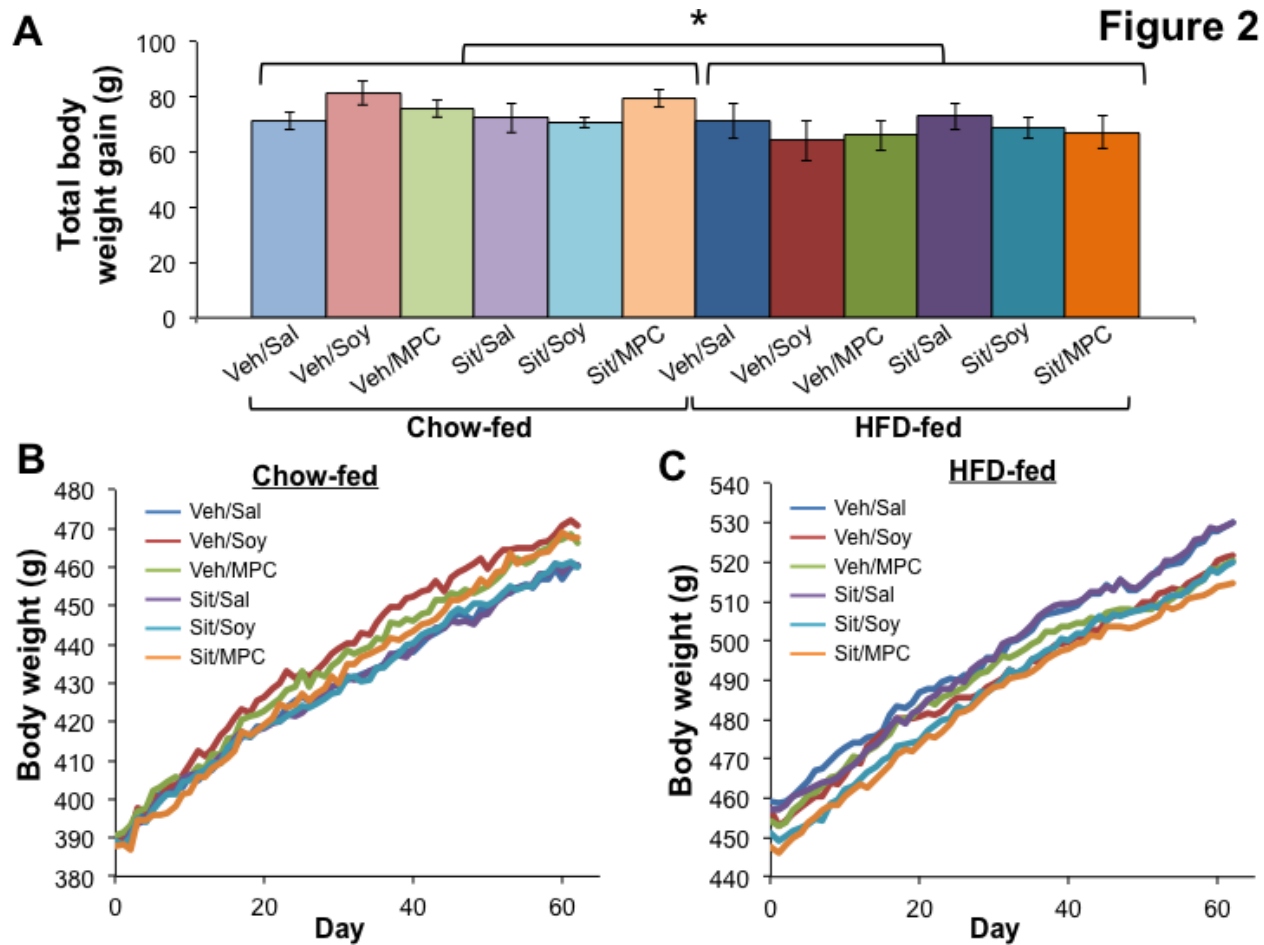


Figure2

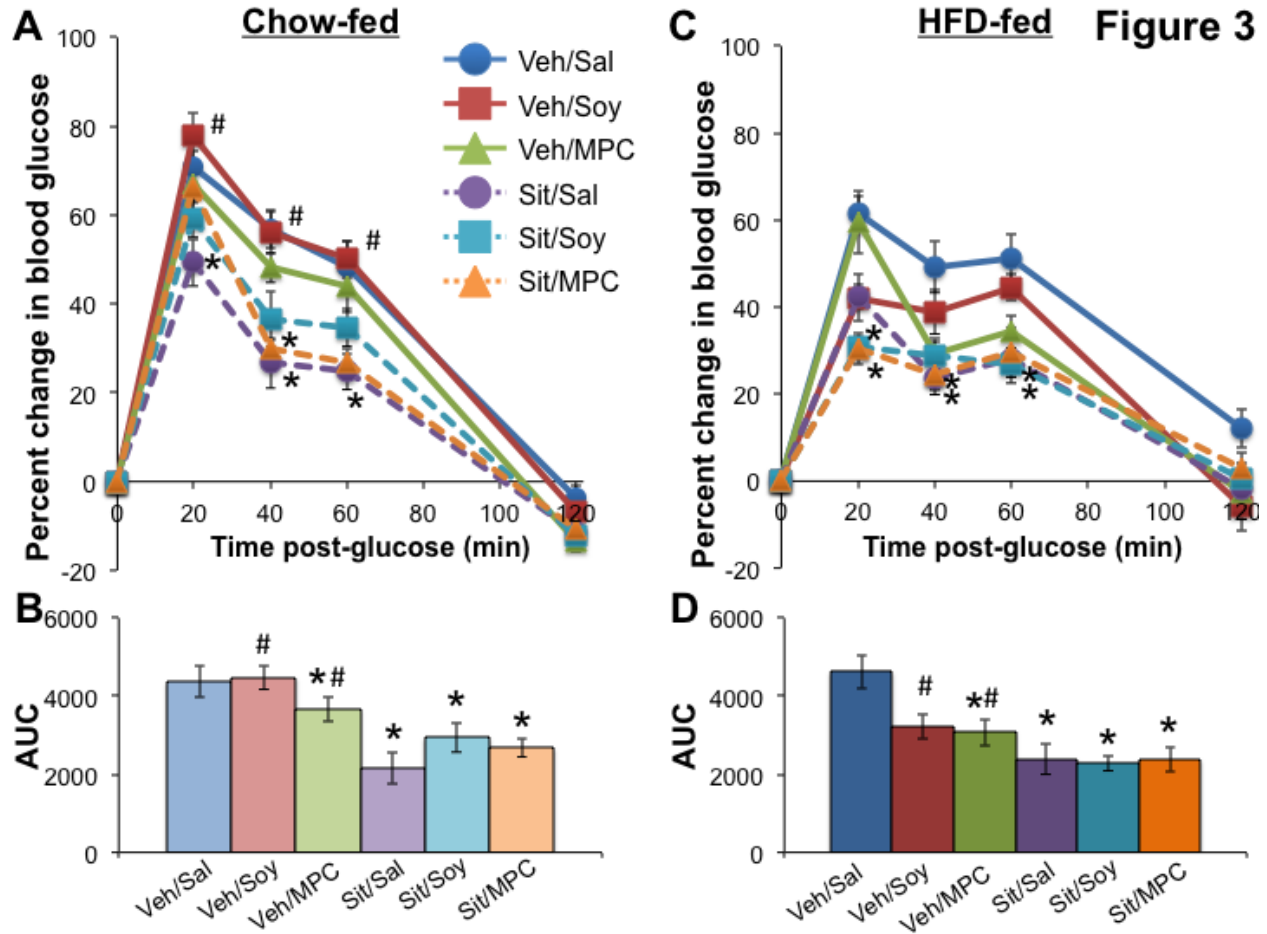


Figure 3

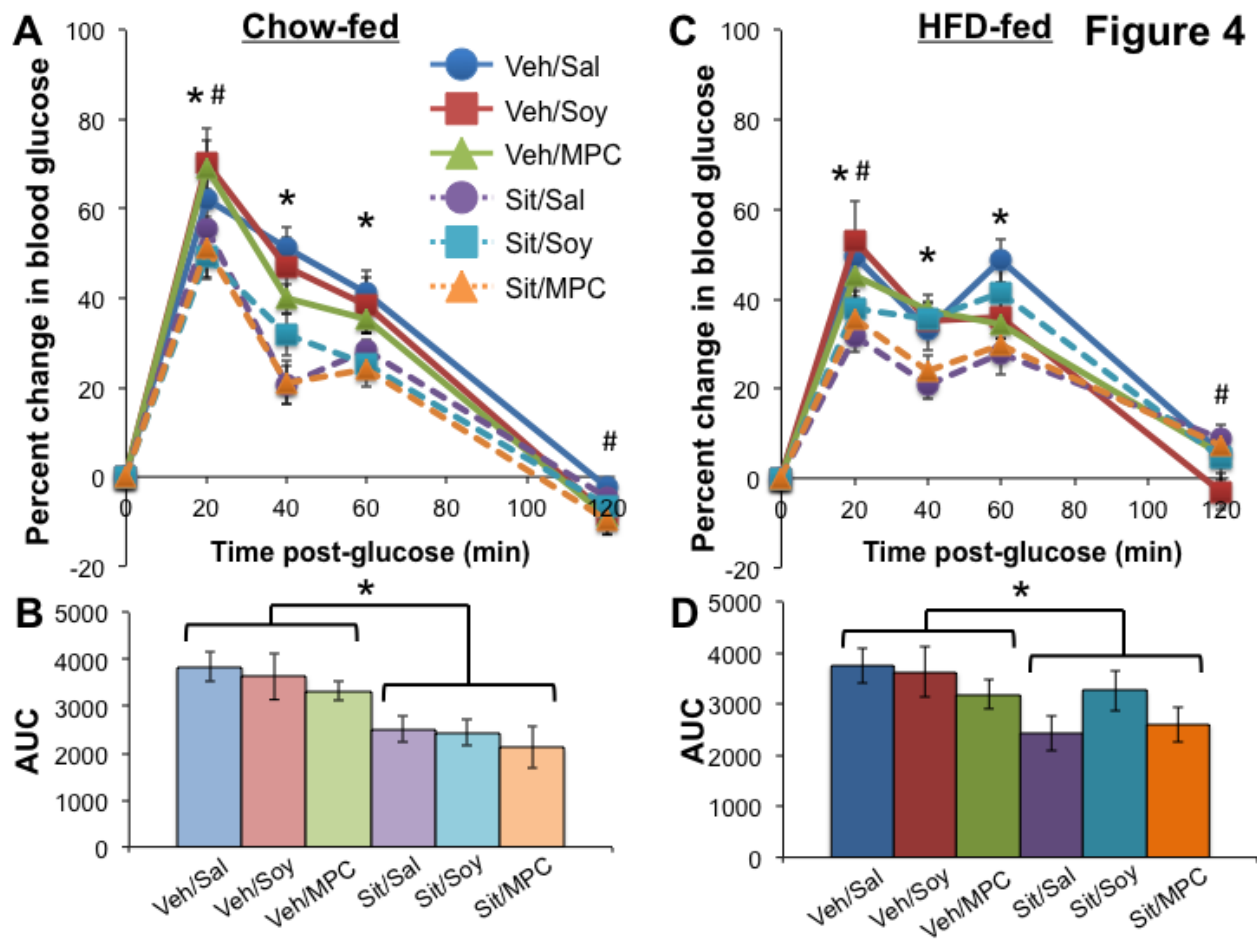


Figure 4

Highlights

- Daily supplementation of dietary protein improves the metabolic effects of GLP-1-based pharmacotherapy in lean and obese rats

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