Acute peripheral GLP-1 receptor agonism or antagonism does not alter energy expenditure in rats after Roux-en-Y gastric bypass

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Abstract: Compared to traditional weight loss strategies, the compensatory decrease in energy expenditure in response to body weight loss is markedly attenuated after Roux-en-Y gastric bypass surgery (RYGB). Because basal and postprandial levels of glucagon-like peptide-1 (GLP-1) are increased after RYGB surgery, and because GLP-1 has been shown to increase energy expenditure, we investigated if increased GLP-1 levels are involved in the alterations in energy expenditure after RYGB. Adult male Wistar rats were randomized for RYGB (n=8) or sham surgery (n=17). Part of the sham-operated rats were food restricted and body weight-matched (n=8) to the RYGB animals. The effects of acute subcutaneous administration of the GLP-1 antagonist Exendin (9-39) (Ex-9, 30 g/kg) or the GLP-1 agonist Exendin-4 (Ex-4, 5 g/kg), respectively, on energy expenditure were tested using indirect calorimetry. We found that Ex-9 increased food intake in RYGB, but not in sham-operated rats. Energy expenditure was lower in RYGB and sham-operated body weight-matched rats compared to sham-operated ad libitum fed rats, but significantly higher in RYGB rats compared to sham-operated body weight-matched rats. There was no effect of Ex-9 treatment on energy expenditure in either group of animals. Similarly, Ex-4 decreased food intake more in RYGB than in sham-operated rats, but Ex-4 did not modulate energy expenditure in any surgical group. We conclude that acute modulation of GLP-1 signaling is not directly involved in altered energy expenditure after RYGB surgery in rats.

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Title: Acute peripheral GLP-1 Receptor Agonism or Antagonism does not alter Energy Expenditure in Rats after Roux-en-Y Gastric Bypass

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1. Introduction

Obesity with its resulting comorbidities has become a major topic in global healthcare and disease prevention [1]. Currently, bariatric surgery is the treatment of choice for obese patients because weight loss maintenance and improvement or even resolution of co-morbidities such as type 2 diabetes mellitus is achieved in many cases [2-5]. The Roux-en-Y Gastric Bypass (RYGB) is the most commonly performed bariatric procedure and can be considered the gold standard in bariatric surgery [6]. Several underlying physiological mechanisms have been identified that potentially contribute to weight loss after RYGB; these mechanisms include reduced hunger and increased satiation [7-9], changes in meal patterns [10], a reduced preference for high fat diet [11-13], alterations in sweet taste function [12-15], as well as absence of a compensatory decrease in energy expenditure [16, 17].

We and others previously reported that body weight loss in rats after RYGB is not associated with the decrease in energy expenditure that is observed with traditional weight loss strategies [16, 17]. This finding was interesting because maintenance of a lower body weight by dietary caloric restriction fails in many obese patients due to compensatory metabolic responses such as a decrease of energy expenditure [18]. The reasons for the absence of decreased energy expenditure after RYGB surgery in rats are unknown, but it has been hypothesized that the increased postprandial release of glucagon-like peptide-1 from the distal small intestine after RYGB may be involved [7, 8, 19]. From all gastrointestinal hormones affected by RYGB, GLP-1 has most consistently been reported to be elevated after RYGB in humans and rats and is thought to be at least partly responsible for the reduction in food intake and the early improvement of glucose tolerance after surgery [4, 5, 7, 8, 19, 20].

GLP-1's effects on food intake have been characterized in numerous studies (e.g., [21-25]), but its role in the control of energy expenditure is less well investigated. However, there are

some studies that suggest an involvement of GLP-1 in energy expenditure. For example, Osaka et al showed a dose-dependent increase in oxygen consumption after intravenous GLP-1 administration [26]. Furthermore, mice lacking the GLP-1 degrading enzyme dipeptidyl peptidase IV (DPP IV) are resistant to high fat diet-induced obesity due to reduced food intake and increased energy expenditure [27].

In this project, we therefore wanted to assess a possible role of the endocrine system and in particular of the satiating gut hormone GLP-1 in energy expenditure after RYGB in rats. We hypothesized that acute peripheral modification of GLP-1 signaling may influence the changes of energy expenditure induced by RYGB.

2. Methods

2.1. Animals

Twenty-five adult male Wistar rats weighing 400 – 450 g preoperatively were allocated to either RYGB (n = 8) or sham-operation (n = 17). After a recovery period of 10 days, sham-operated animals were randomly divided into two groups: sham-operated rats with no dietary manipulation (n=9, ad libitum fed shams weighing 441 ± 16 g 10 days after surgery) and food-restricted shams whose postoperative weight was matched to the weight of RYGB rats (body weight-matched shams (BWm) weighing 433 ± 20 g 10 days after surgery). BWm shams received as much food daily as was necessary to maintain a similar body weight as the RYGB rats. Based on experiences from previous studies [16], rats were given approximately 14 g of standard chow in the beginning of food restriction period. This amount of food was offered at dark onset and readjusted every third day depending on the body weight development.

2.2. Housing

All animals were individually housed under an artificial 12 hour / 12 hour light-dark cycle (lights off at 1500) and at a room temperature of 21±2ºC unless otherwise stated. Water and standard chow were available ad libitum. All experiments were approved by the Veterinary Office of the Canton Zurich, Switzerland.

2.3. Surgery

Anesthesia was induced in a chamber filled with 5% isoflurane in oxygen (1 liter per min). After an adequate depth of anesthesia was achieved, rats were shaved from sternum to pelvis followed by disinfection with betadine scrub. Rats were then placed in supine position on a heating pad and positioned in a nose cone to maintain anesthesia (2-4% isoflurane in oxygen, 0.5 liter per min) for the duration of surgery. Operations were performed as previously described [28]. Briefly, a midline incision of approximately 4 cm starting just below the xyphoid process was performed. For the RYGB procedure, the small bowel was transected approximately 20 cm distal to the pylorus of the stomach creating a proximal and distal end of small bowel. The proximal end being still continuous with the remaining portion of the stomach constituted the biliopancreatic limb and was anastomosed to the ileum approximately 25-30 cm from the caecum creating the common channel. For formation of the gastric pouch, the stomach was transected approximately 5 mm below the gastro-esophageal junction creating a gastric pouch of a size of no more than 2-3% of original stomach size. The Roux-en-Y reconstruction was completed by connecting the distal end of the small bowel to the gastric pouch leading to formation of the alimentary limb. One single RYGB procedure lasted approximately 70 min. For sham operations, an anterior gastrotomy and a jejunotomy with subsequent closures were performed. One single sham procedure lasted approximately 30 min. The abdominal wall and the skin were closed in layers after both operations.

2.4. Indirect calorimetry

Measurements were conducted in an open circuit calorimetry system (AccuScan Inc., USA) as previously described [29, 30]. Briefly, rats were individually housed in Plexiglas airtight metabolic cages (41x41x31 cm) on a layer of wood shavings under the same light and temperature conditions as described above. Water and standard powder chow (GLP3433,

Provimi Kliba Ag, Switzerland) were available ad libitum, unless otherwise stated. Food and water intake were measured continuously. Physical activity was monitored by a 3-dimensional array of infrared light beams and sensors. Thus, the activity data provided represent a relative measure of locomotor activity of the rats (movement/hour). The activity data do not relate to an absolute measurement of distance moved or to a spatial position.

Energy expenditure was calculated for each 30 second sample according to Weir using the following equitation: total energy expenditure (kcal/h) = $3.9xV(O_2)L/h+1.1xV(CO_2)L/h$ [29, 30]. The respiratory quotient was defined as the quotient of CO_2 production and O_2 consumption.

2.5. Experimental design

Measurements of energy expenditure in all groups were conducted between postoperative week 5 and 11. Experiments were performed in a randomized, saline-controlled, crossover manner. About one week before treatment, all rats were placed for at least two days in the metabolic chambers for adaptation.

First, the potency of endogenous GLP-1 to alter energy expenditure after RYGB was tested with the GLP-1 receptor antagonist Exendin (9-39) (Ex-9); we allowed two days of wash-out between crossover days, i.e. between peptide and saline injection, respectively. Tests were performed similar to Williams et al [25]. As shown in Figure 1, rats were deprived of chow, but not water, 8 h prior to dark onset (0700 h). At 1100 h, 30 µg/kg Ex-9 or the saline vehicle (1 ml/kg) was injected subcutaneously and measurement of energy expenditure was started. One hour later chow was provided ad libitum again (1200 h, i.e. 3h prior to dark onset).

Second, the potency of exogenous GLP-1 to alter energy expenditure after RYGB was tested with the GLP-1 agonist Exendin-4 (Ex-4) with three days of wash-out between peptide and saline. As demonstrated in Figure 2, rats were deprived of chow, but not water, 6 h prior to dark onset (0900 h). At 1400 h, 5 µg/kg Ex-4 or the saline vehicle (1 ml/kg) was injected subcutaneously and measurement of energy expenditure was started. One hour later, i.e. at dark onset, chow was provided ad libitum again (1500 h). The experiments using Ex-4 were performed about 4 weeks after the experiments using Ex-9. Dose and time course of Ex-4 administration was chosen based on an unpublished pilot experiment that showed a significant decrease in chow intake ($p<0.05$) of intact male Wistar rats (n=12) up to 24h after injection.

2.6. Statistical analysis

A one-way ANOVA was performed to analyze average daily food intake as well as differences in body weight between groups. Following a significant F ratio, a Bonferroni post hoc test was used to determine differences between groups. Differences in energy expenditure, food intake, physical activity and respiratory quotients after Ex-9 and Ex-4 treatments were analyzed with a two-way, repeated measures (RM) group (between subjects) x treatment (within subjects) analysis of variance (ANOVA) followed by Bonferroni post-hoc tests for each treatment group when there was a significant group x treatment interaction. Significance was established at $P \leq 0.05$ for all statistical sets and data are reported as mean ± SEM.

3. Results

3.1. Body weight

Figure 3 shows the development of body weight for all three groups. Body weight was significantly lower in RYGB rats compared to the sham-operated ad libitum fed group from postoperative day 6 (sham ad lib: 441 ± 16 g vs. RYGB: 383 ± 16 g, p=0.020). In postoperative week 14, the difference in body weight was about 240 g (sham ad lib: 628 ± 34 g vs. RYGB: 389 ± 81 g, p=0.012). After a short period of post-surgical weight loss, shams ad libitum fed constantly gained weight for the rest of the study. In contrast, RYGB animals lost 20% of their preoperative weight by postoperative week 2 with a weight nadir of 360 ± 45 g. Until postoperative week 5, RYGB rats then regained a moderate amount of body weight followed by a weight plateau around 380 and 390 g throughout the rest of the observation period. Food restriction started ten days after surgery for sham-operated BWm rats (n=8). There was no significant difference in body weight between the RYGB group and the food restricted sham BWm rats at postoperative week 4 (sham BWm: 405 ± 13 g vs. RYGB: 379 ± 43, p=0.56) and thereafter.

3.2. Average daily food intake

Figure 4 shows the average daily food intake for all three groups throughout the entire observation period from postoperative week 0 – 14. Daily food intake was consistently lower after RYGB (sham ad lib: 29.6±0.9 g vs. RYGB: 25.5±0.8 g, p<0.01). BWm shams required significantly less food than RYGB animals to maintain the same level of body weight (sham BWm: 14.4±0.04 g vs. RYGB: 25.5±0.8 g, p<0.001).

3.3. Effect of treatment with GLP-1 receptor antagonist Exendin (9-39)

3.3.1. Energy Expenditure

Repeated measures (RM) two-way ANOVA revealed a main effect of surgical group for mean 24 hour energy expenditure ($p<0.001$); energy expenditure after RYGB was significantly higher compared to sham-operated BWm controls, but significantly lower in comparison to sham-operated ad libitum fed rats after both saline and Ex-9 treatment (Figure 5A). However, there was no difference in 24 hour energy expenditure when comparing the corresponding surgery groups that were treated with saline or Ex-9, respectively; RM two-way ANOVA showed no main effect of treatment for saline or Ex-9 treatment, respectively ($p=0.79$). There was also no significant group x treatment interaction ($p=0.72$).

During the first hour after Ex-9 and saline injection with no food available (1100 – 1200), RM two-way ANOVA revealed a main effect of surgical group ($p<0.001$). Energy expenditure tended to be higher after RYGB in comparison to sham-operated BWm controls, but this did not attain statistical significance. However, energy expenditure after RYGB was significantly lower than after sham-operation and ad libitum food; this was observed after both saline and Ex-9 treatment, respectively (Figure 5B). Similar to the 24h values, Ex-9 had no effect on energy expenditure during the first hour after treatment in any surgery group, i.e. there was no main effect of treatment for saline or Ex-9, respectively ($p=0.88$). There was also no significant group x treatment interaction ($p=0.58$).

For the first two hours after food was made available ad libitum again (1200 – 1400), RM two-way ANOVA showed no main effect of surgical group ($p=0.32$) or treatment ($p=0.87$) for energy expenditure after Ex-9 and saline injection, respectively; there was also no group x treatment interaction ($p=0.72$) (Figure 5C). Figure 5D shows the time course of 24 hour

energy expenditure after Ex-9 and saline treatment for all three groups. The values of the RM two-way ANOVA for energy expenditure after Ex-9 treatment are given in Table 1.

3.3.2. Food intake, physical activity and Respiratory quotient (RQ)

Food intake was analyzed for the first two hours once food was made available ad libitum again (1200 – 1400). Here, RM two-way ANOVA showed a main effect of surgical group for mean food intake after Ex-9 and saline injection, respectively ($p<0.001$) (Figure 6A). While there was no main effect of treatment for mean food intake ($p=0.13$), RM two-way ANOVA revealed a significant group x treatment interaction for mean food intake after Ex-9 or saline injection ($p=0.004$), respectively, indicating that the Ex-9 effect differed significantly between the surgical groups. RYGB rats, but not sham-operated rats ate significantly more after Ex-9 than after saline (RYGB: saline 3.5 ± 0.4 g vs. Ex-9 4.9 ± 0.6 g, $p<0.01$); in fact, sham-operated rats ate 25% less after Ex-9 compared to saline, and RYGB rats ate 65% more after Ex-9 during the first two hours of food access (Figure 6B; sham: 73.7 ± 13.2 % vs. RYGB: 165.1 ± 25.3 %, $p<0.05$). Further, sham-operated BWm rats showed the highest food intake in this two hour period, which is clearly the result of being conditioned to feeding regime with long periods of fasting before a limited amount of food was offered at dark onset. Thus, it was not surprising that we did not observe an effect of Ex-9 treatment in this group of rats.

Repeated measures (RM) two-way ANOVA further revealed a main effect of surgical group for the physical activity during the first two hours after food return ($p<0.001$), with sham-operated BWm controls showing more movements per hour than sham-operated ad libitum fed or RYGB operated rats (Figure 6C). However, there was no main effect for treatment ($p=0.87$) and no significant group x treatment interaction ($p=0.72$). Finally, RM two-way ANOVA showed a significant main effect of surgical group for the respiratory quotient during

the first two hours after food return ($p<0.05$), but there was no main effect for treatment and no group x treatment interaction (Figure 6D). Sham-operated BWm controls had significantly higher respiratory quotients than sham-operated ad libitum fed and RYGB operated rats, irrespective of Ex-9 or saline injection. The p values of the RM two-way ANOVA for food intake, physical activity and respiratory quotient after Ex-9 treatment are given in Table 1.

3.4. Effect of GLP-1 receptor agonist Exendin-4

3.4.1. Energy Expenditure

Repeated measures (RM) two-way ANOVA revealed a main effect of surgical group for mean 24 hour energy expenditure ($p<0.001$) with energy expenditure after RYGB being significantly higher compared to sham-operated BWm controls, but significantly lower in comparison to sham-operated ad libitum fed rats after both saline and Ex-4 treatment (Figure 7A). However, there was no Ex-4 effect in any surgical group; RM two-way ANOVA showed no main effect of treatment for saline or Ex-4, respectively ($p=0.69$) and no significant group x treatment interaction ($p=0.83$).

Similar to the 24h values, RM two-way ANOVA revealed a main effect of surgical group on energy expenditure during the first hour after Ex-4 and saline when no food was available (1400 – 1500) ($p<0.001$). Energy expenditure tended to be higher after RYGB compared to sham-operated BWm controls after saline or Ex-4, respectively ($p>0.05$), but lower than after sham-operation and ad libitum food (Figure 7B).

Overall, we found a main effect of treatment during the first hour after Ex-4 and saline, respectively, when rats were fasted ($p<0.001$); Ex-4 reduced energy expenditure in all surgical groups of rats, but differences were only significant in sham-operated BWm rats (sham:

saline: 2.5 ± 0.2 kcal/h vs. Ex-4: 2.4 ± 0.1 kcal/h, p>0.05; sham BWm: saline: 1.9 ± 0.2 kcal/h vs. Ex-4: 1.5 ± 0.03 kcal/h, p<0.01; RYGB: saline: 2.1 ± 0.1 kcal/h vs. 1.9 ± 0.1 kcal/h, p>0.05). There was no significant group x treatment interaction (p=0.17).

Repeated measures (RM) two-way ANOVA further showed a main effect of surgical group (p<0.001), but not treatment (p=0.16), on energy expenditure during the dark phase after Ex-4 and saline injection (Figure 7C), respectively, but there was also no group x treatment interaction (p=0.48). Similar to the complete 24 h period, energy expenditure was higher after RYGB than in sham-operated BWm controls during the dark phase, but significantly lower in comparison to sham-operated ad libitum fed rats after both saline and Ex-4 treatment. Figure 7D shows the time course of 24 hour energy expenditure after Ex-4 and saline treatment for all three surgical groups. The values of the two-way ANOVA for energy expenditure after Ex-4 treatment are given in Table 2.

3.4.2. Food intake, physical activity and Respiratory quotient (RQ)

Repeated measures (RM) two-way ANOVA showed a main effect of the surgical group for mean food intake during the dark phase following Ex-4 or saline injection, respectively (p<0.001) (Figure 8A). There was also a main effect of treatment for mean food intake (p<0.001) and a significant group x treatment interaction (p<0.001). Sham-operated and RYGB rats reduced their food intake after Ex-4 administration (sham: saline: 25.3 ± 0.5g vs. Ex-4: 20.8 ± 0.8g, p<0.001; RYGB: saline: 18.9 ± 2.2g vs. Ex-4: 11.6 ± 1.2g, p=0.004). The Ex-4 induced reduction in dark phase eating was significantly stronger in RYGB than in sham-operated rats (Figure 8B [saline control = 100%]; sham: 82.3 ± 2.3 % vs. RYGB: 64.4 ± 5.9 %, p=0.008).

Repeated measures (RM) two-way ANOVA further revealed no significant differences in physical activity and respiratory quotient between the three groups during the dark phase after Ex-4 and saline injection, respectively (Figure 8C and D). However, there was a main effect of treatment during the first hour after Ex-4 and saline, respectively, when rats were fasted ($p<0.01$); Ex-4 reduced activity in all surgical groups of rats, but differences were only significant in sham-operated BWm rats (sham: saline: 86.78 ± 18.6 movements/h vs. Ex-4: 54.0 ± 6.0 movements/h, $p>0.05$; sham BWm: saline: 171.8 ± 34.5 movements/h vs. Ex-4: 64.4 ± 10.4 movements/h, $p<0.05$; RYGB: saline: 125.5 ± 32.4 movements/h vs. 61.1 ± 11.4 movements/h, $p>0.05$). The values of the RM two-way ANOVA for food intake, physical activity and respiratory quotient after Ex-4 treatment are given in Table 2.

4. Discussion

We demonstrated that acute subcutaneous administration of a low dose of the GLP-1 antagonist Exendin (9-39) (Ex-9, 30 µg/kg) or the GLP-1 agonist Exendin-4 (Ex-4, 5 µg/kg) did not alter energy expenditure in RYGB or sham-operated rats. Overall energy expenditure was lower in RYGB compared to sham-operated ad libitum fed rats, but energy expenditure was significantly higher in RYGB rats compared to their body weight matched counterparts. Against our hypothesis, we found that the administration of Ex-9 or Ex-4, respectively, at doses that affected eating had no measurable effect on energy expenditure in either group of animals.

Consistent with this study, we and others had previously reported that body weight loss in rats after RYGB was not associated with the decrease in energy expenditure that is usually observed with traditional weight loss strategies [16, 17, 31, 32]. Such compensatory metabolic responses with a decrease in the resting metabolic rate are believed to be the main reason why maintenance of a lower body weight only by caloric restriction fails in the majority of obese patients [18]. The lack of this compensatory decrease in energy expenditure is therefore an important and promising finding that requires further investigation in order to detect the underlying mechanisms.

We and others had reported that the RYGB procedure leads to an increased postprandial release of GLP-1 in humans and rats. GLP-1's effects on food intake have been characterized in numerous studies (e.g., [21, 23-25, 33]), and elevated GLP-1 is thought to be at least partly responsible for the reduction in food intake after RYGB surgery. Several studies point to an additional role of GLP-1 in the regulation of energy balance by directly influencing energy expenditure. For example, Osaka et al. [26] showed a dose-dependent increase in oxygen consumption after intravenous GLP-1 administration; this effect was thought to be mediated

by the lower brainstem and to require the integrity of the sympathoadrenal system. In addition, Lockie et al. [34] reported an increase in brown adipose tissue (BAT) thermogenesis after acute central injection of GLP-1, which was associated with increased activity of sympathetic fibers that innervate BAT. Furthermore, mice lacking the GLP-1 degrading enzyme dipeptidyl peptidase IV (DPP IV) are resistant to high fat diet-induced obesity because of reduced food intake and increased energy expenditure [27]. However, the latter results have to be interpreted with caution because DPP IV not only degrades GLP-1, but is also involved in the metabolism of many other peptides including PYY (which is activated to PYY3-36), oxyntomodulin (OXM) and glucagon-like peptide-2 (GLP-2). Like GLP-1, postprandial levels of these hormones are increased after RYGB surgery [8, 19, 35-37], and several studies show an involvement of PYY [38] and OXM [39, 40] in the control of energy expenditure.

In the present study, we found no evidence that acute modulation of GLP-1 signaling affects the changes in energy expenditure seen after RYGB, even though we saw the expected effects of Ex-9 (increase) and Ex-4 (decrease) on eating. In fact, we provide evidence that at least under our experimental conditions enhanced GLP-1 signaling contributes to the eating inhibitory effect of RYGB, because Ex-9 increased eating only in RYGB but not in sham-operated rats. We also showed that Ex-4 decreased eating more in RYGB than in sham-operated rats. Hence, despite higher baseline and postprandial GLP-1 levels [19, 41, 42], RYGB rats exhibit no desensitization to the effect of exogenous GLP-1 or its agonists, respectively, which is in consistence with previous reports [43].

Considering our findings that do not support a role of GLP-1, we have to presume that other mechanisms need to be considered in order to explain the absence of a compensatory decrease in energy expenditure after RYGB surgery in rats. One possible mechanism consists of a higher energy requirement due to the significant hypertrophic changes of the small intestine after RYGB surgery [16, 37]. In fact, increased GLP-2 levels after RYGB may be responsible for mucosal hypertrophy, and this may help to limit malabsorptive consequences of the RYGB surgery [37]. GLP-2 has well-characterized, positive effects on epithelial proliferation particularly in the small intestine [44] leading to an increased resorptive surface by enhanced crypt cell proliferation and reduced apoptosis of enterocytes [45, 46]. Because the gastrointestinal tract is a metabolically very active organ [47], its hypertrophy is likely to contribute to the increase in energy expenditure after RYGB especially in response to a meal.

Differences in energy expenditure between RYGB and control rats might also be related to alterations in postprandial (or diet-induced) thermogenesis [16], even though they seem to be GLP-1 independent. Diet-induced thermogenesis is mediated in part by BAT [48, 49], and it has recently been found that BAT in adult humans correlated negatively with BMI, fasting glucose levels [50] and non-alcoholic fatty liver disease [51]. However, using 18F-FDG PET/CT imaging for measurement of the metabolic activity of brown adipose tissue in RYGB and sham-operated rats, Hankir et al. were unable to demonstrate an increase in BAT activity, suggesting that other mechanisms are involved to explain the increased energy expenditure after RYGB [52]. Furthermore, there was no difference in the UCP-1 mRNA content of BAT between the two groups [52].

Our study has several limitations. First, we decided to study acute effects of GLP-1 receptor modulation on energy expenditure after RYGB surgery without investigating chronic effects. This experimental approach was based on previous results demonstrating that our indirect calorimetry system allowed us to specifically detect short-term changes in energy expenditure [29, 30]. However, there is some evidence that the acute administration of GLP-1 agonists decreases energy expenditure, potentially as a direct consequence of the reduction in food intake (53). Consistently, Ex-4 treatment led to a decrease in fasting energy expenditure in our study, which was associated with reduced physical activity probably due to reduced food-seeking behavior. Because the acute effects of a single-dose injection may be very different from the effects of chronic alterations in hormone signaling as observed after RYGB surgery, it would be important to see whether chronic GLP-1 receptor agonism or antagonism does have an effect on energy expenditure after RYGB or not, e.g. by using subcutaneous mini-pump systems. It would also be interesting to compare the effects of peripheral and central GLP-1 receptor modulation after RYGB surgery. Second, we only performed single dose studies. Since the mechanisms underlying the control of energy expenditure and food intake, respectively, by GLP-1 may not be identical, the absence of an acute effect of the respective compounds on energy expenditure could still be due to an ineffective dose, even though we observed the expected effects on food intake, However, the decrease in fasting energy expenditure after Ex-4 injection suggests that the acute administration of a higher dose is unlikely to yield results that would support our hypothesis, i.e. an effect consistent with the idea that elevated GLP-1 post RYGB contribute to the increase in energy expenditure. Third, endogenous GLP-1 levels were not routinely measured

in our RYGB rat model. However, we have previously demonstrated increased postprandial GLP-1 levels in this model on several occasions [14, 43].

In summary, our data suggest that acute modulation of GLP-1 signaling is not directly involved in the altered energy expenditure after RYGB surgery in rats. The underlying mechanisms leading to the changes in energy expenditure in RYGB rats therefore remain unclear. It seems likely that altered energy expenditure is caused by the combined effects of several different factors rather than by just one specific gut hormone.

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Tables

Table 1

	Surgical group	Treatment group	Interaction
24 h Energy Expenditure	F(2,22)=32.8, p<0.001	F(1,22)=0.7, p=0.79	F(2,22)=0.34, p=0.72
1 h Energy Expenditure (1100 – 1200, no food)	F(2,22)=11.74, p<0.001	F(1,22)=0.02, p=0.88	F(2,22)=0.34, p=0.58
2 h Energy Expenditure (1200 – 1400, with food)	F(2,22)=1.21, p=0.32	F(1,22)=0.03, p=0.87	F(2,22)=0.34, p=0.72
2h Food intake (1200 – 1400)	F(2,21)=457.3, p<0.001	F(1,21)=2.47, p=0.13	F(2,21)=7.46, p=0.004
2h Physical activity (1200 – 1400, with food)	F(2,21)=36.8, p<0.001	F(1,21)=0.03, p=0.87	F(2,21)=0.34, p=0.72
2h Respiratory Quotient (1200 – 1400, with food)	F(2,21)=5.3, p=0.013	F(1,21)=0.15, p=0.71	F(2,21)=1.35, p=0.28

Table 2

	Surgical group	Treatment group	Interaction
24 h Energy Expenditure	F(2,22)=17.8, p<0.001	F(1,21)=0.15, p=0.69	F(2,22)=0.19, p=0.82
1 h Energy Expenditure (1400 – 1500, no food)	F(2,22)=9.8, p<0.001	F(1,22)=15.6, p<0.001	F(2,22)=1.93, p=0.17
Energy Expenditure (dark phase, with food)	F(2,22)=22.02, p<0.001	F(1,22)=2.1, p=0.16	F(2,22)=0.75, p=0.48
Food intake (dark phase)	F(2,21)=29.70, p<0.001	F(1,21)=52.73, p<0.001	F(2,21)=16.92, p<0.001
Physical activity (dark phase, with food)	F(2,22)=1.24, p=0.31	F(1,22)=7.66, p=0.011	F(2,22)=1.16, p=0.33
1 h Physical activity (1400-1500, no food)	F(2,22)=2.84, p=0.08	F(1,22)=12.80, p<0.01	F(2,22)=1.31, p=0.29
Respiratory Quotient (dark phase, with food)	F(2,22)=0.37, p=0.695	F(1,22)=4.88, p=0.038	F(2,22)=1.05, p=0.37

1

2

Table 1: RM two-way ANOVA values for energy expenditure, food intake, physical activity and respiratory quotient during 24h or the first 3 hours and after Ex-9 or saline treatment, respectively, as a function of surgical group treatment.

Table 2: RM two-way ANOVA values for 24 hour energy expenditure as well as for energy expenditure, food intake, physical activity and respiratory quotient during the dark phase after Ex-4 and saline treatment, respectively, as a function of surgical group treatment.

Figures

Figure 1

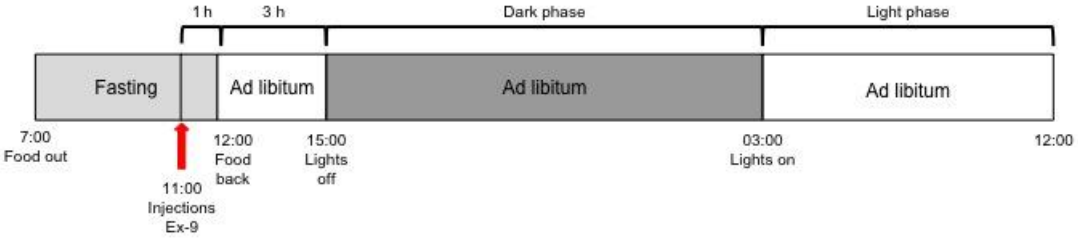


Figure 2

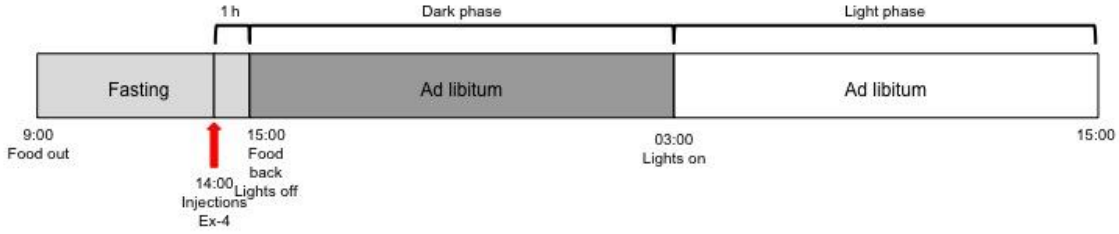
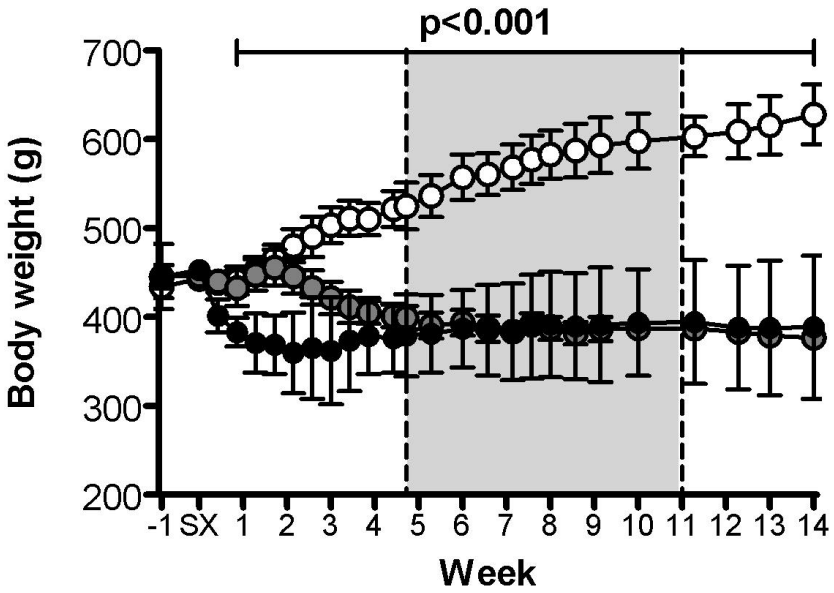


Figure 3



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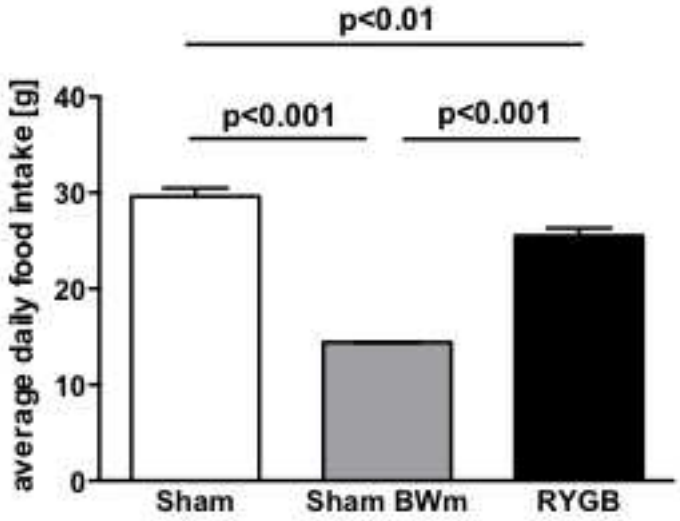

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3 Figure 5

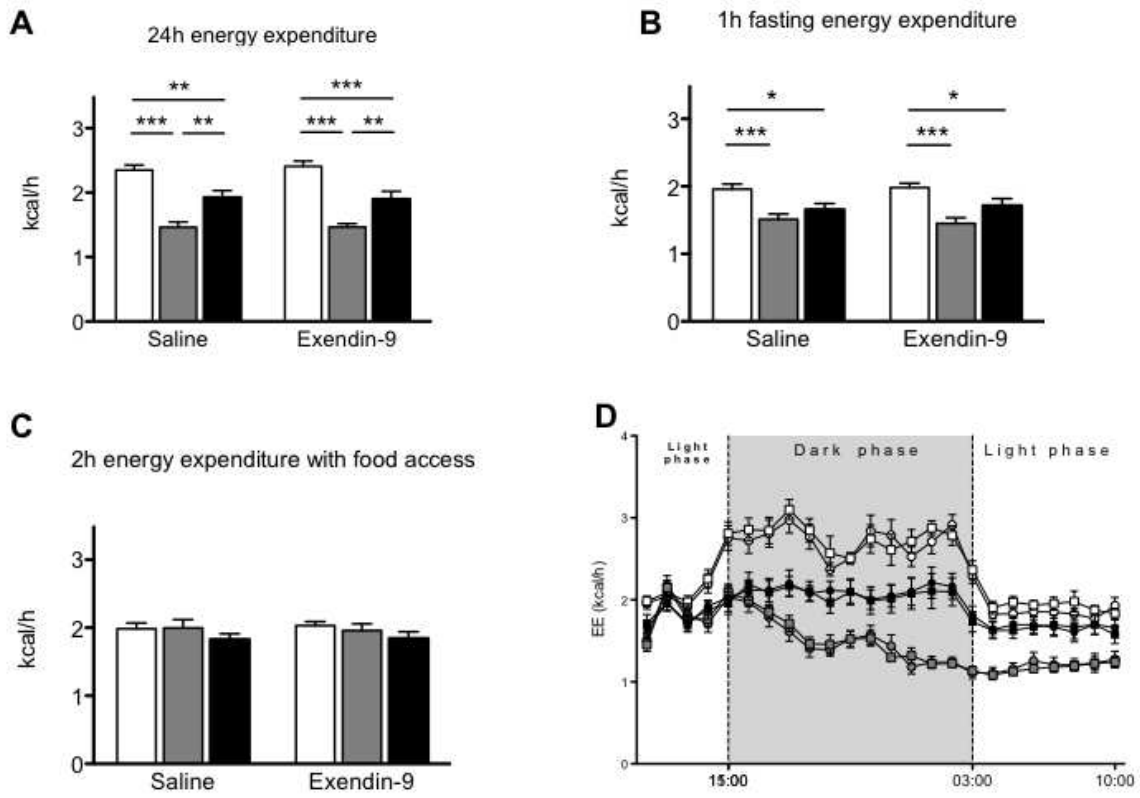

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Figure 6

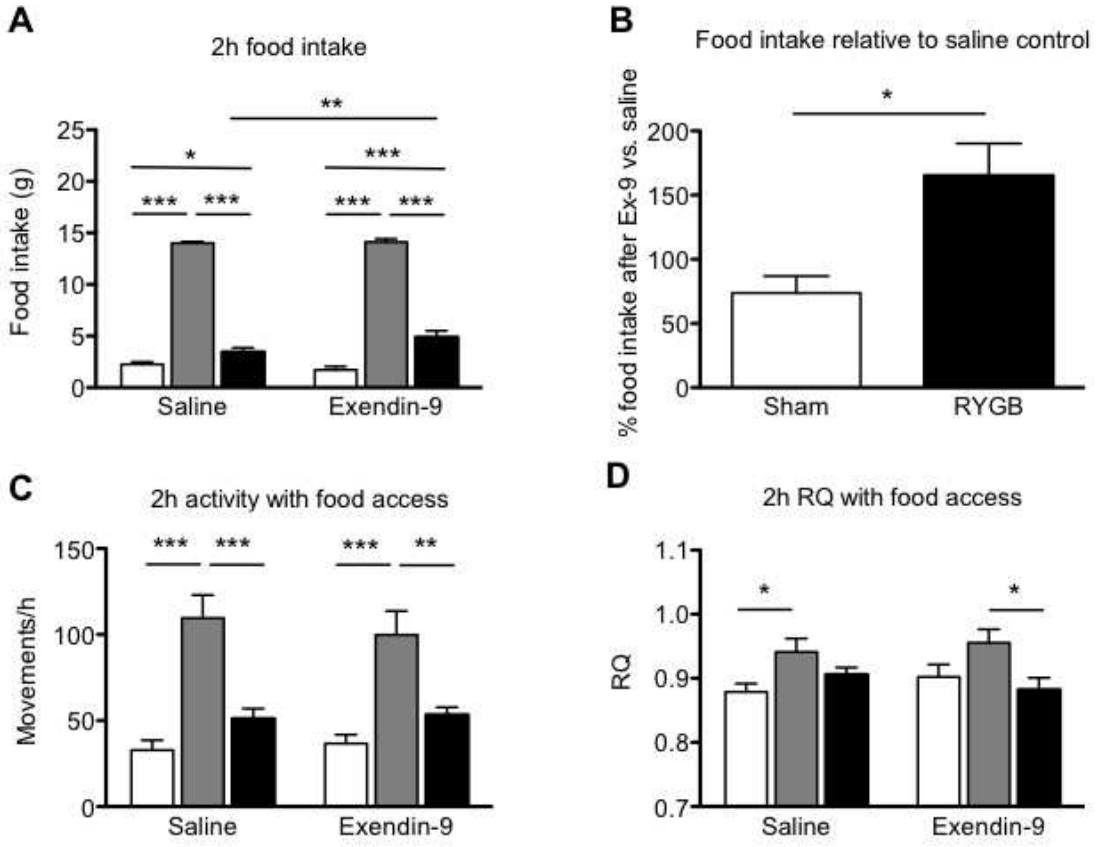
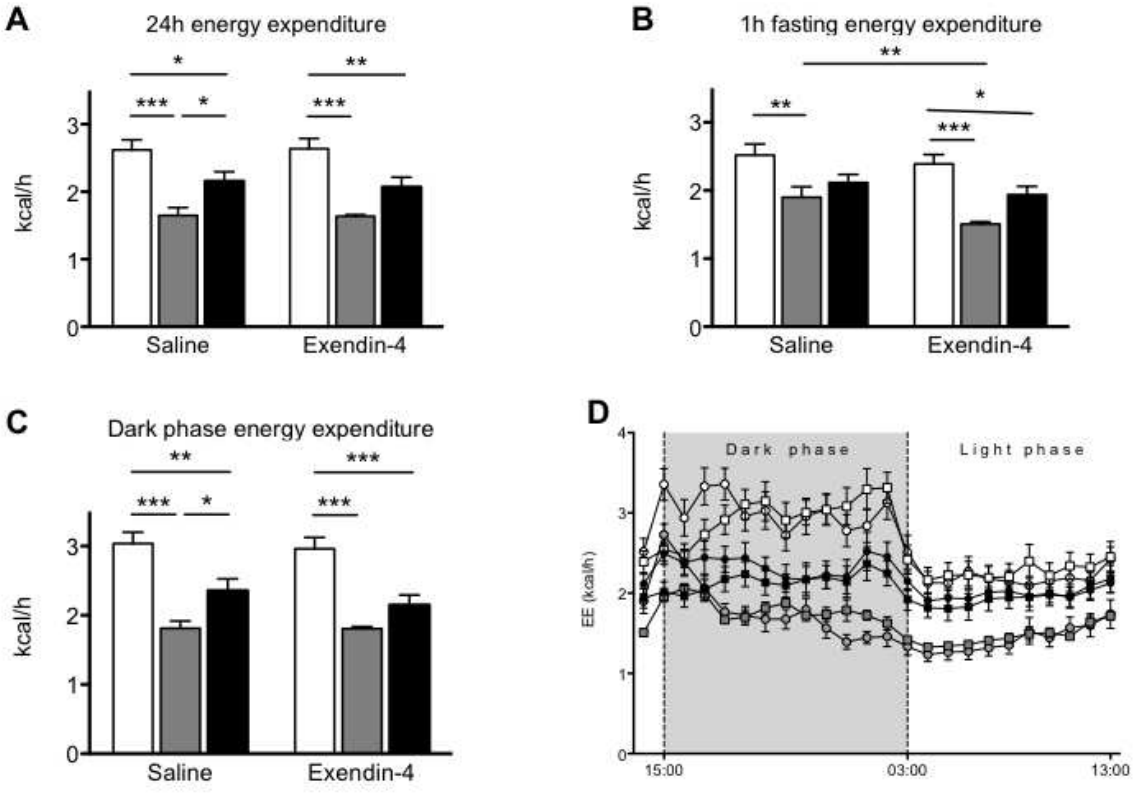
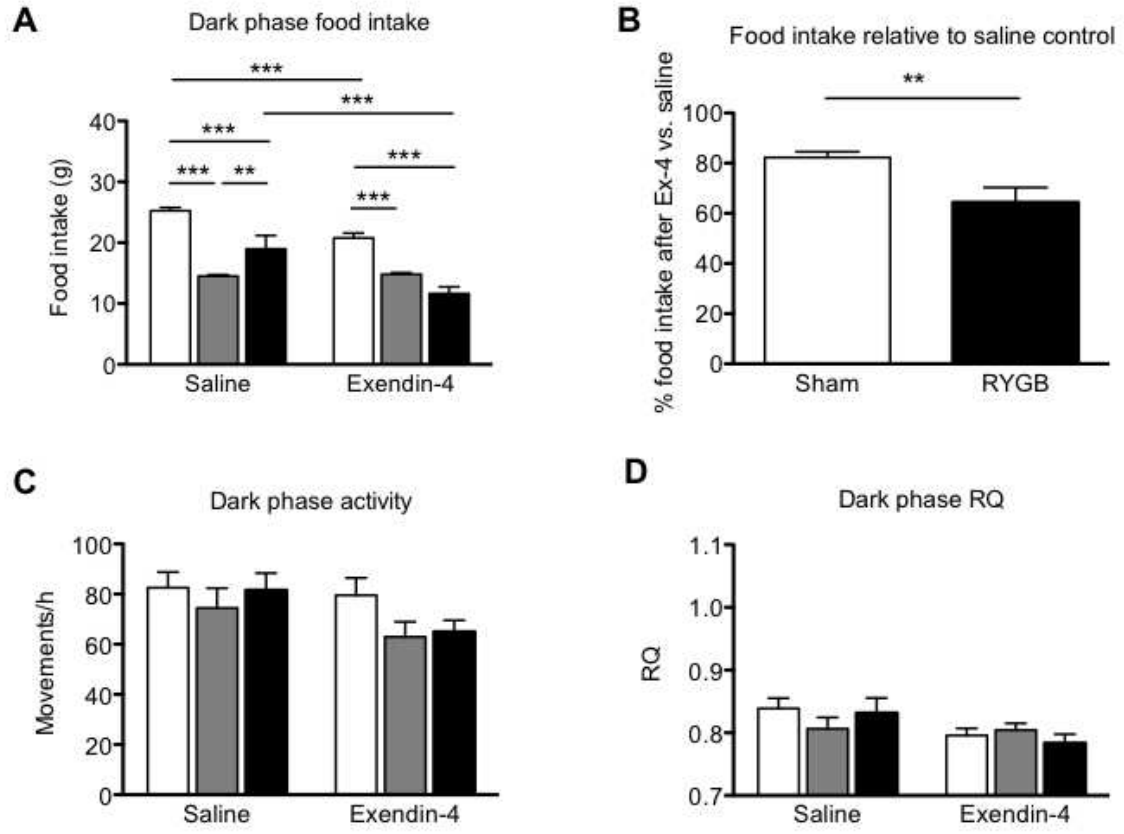


Figure 7



1 Figure 8


A
Dark phase food intake
Food intake (g)
0
10
20
30
40
***
***
***
**
***
***
Saline
Exendin-4
B
Food intake relative to saline control
% food intake after Ex-4 vs. saline
0
20
40
60
80
100
**
Sham
RYGB
C
Dark phase activity
Movements/h
0
20
40
60
80
100
Saline
Exendin-4
D
Dark phase RQ
RQ
0.7
0.8
0.9
1.0
1.1
Saline
Exendin-4

2

Figure 1: Schematic illustration of the experimental protocol to analyze the treatment effect of GLP-1 receptor antagonist Exendin (9-39). Rats were deprived of food 8 h prior to dark onset (0700 h). At 1100 h, 30 µg/kg Ex-9 or the saline vehicle (1 ml/kg) was injected subcutaneously. One hour later chow was provided ad libitum again (1200 h).

Figure 2: Schematic illustration of the experimental protocol to analyze the treatment effect of GLP-1 receptor antagonist Exendin-4. Rats were deprived of food 6 h prior to dark onset (0900 h). At 1400 h, 5 µg/kg Ex-4 or the saline vehicle (1 ml/kg) was injected subcutaneously. One hour later, chow was provided ad libitum again (1500 h).

Figure 3: Body weight change for the sham-operated rats ad libitum fed (white circles, n=9), sham-operated BWm rats (grey circles, n=8) and for RYGB rats (black circles, n=8). E Body weight was significantly lower in RYGB rats compared to the sham-operated ad libitum fed group from postoperative day 6 as indicated ($p<0.001$). Energy expenditure measurements were performed between postoperative week 5 and 11as indicated. Data are shown as mean values ± SEM.

Figure 4: Average daily food intake over 14 weeks for sham-operated ad libitum fed rats (n=9, white column), for sham-operated BWm rats (n=8, grey column) and for RYGB rats (n=8, black column). Data are shown as mean values ± SEM.

Figure 5: **A** Mean 24h Energy Expenditure **B** Average Energy Expenditure during the first hour after Exendin (9-39) (30 µg/kg) and saline s.c. administration without food available **C** Average Energy Expenditure during the first 2h after food return after Exendin (9-39) (30 µg/kg) and saline s.c. administration **D** Time course of 24h Energy Expenditure after Exendin

(9-39) (30 µg/kg) and saline s.c. administration. In **A**, **B** and **C** sham-operated rats ad libitum fed are shown by white columns, sham-operated BWm rats by grey columns and RYGB rats by black columns. In **D** a similar colour code is used with squares representing Exendin (9-39) and circles representing saline treated rats. As two-way ANOVA revealed a significant F ratio, a Bonferroni post hoc test was used to determine differences between groups (*** = $p < 0.001$, ** = $p < 0.01$). Data are shown as mean values ± SEM.

Figure 6: A Average spontaneous food intake during the first 2h when food was available **B** Percentage change in food intake after Ex-9 treatment compared to saline treatment **C** Average physical activity during the first 2h after food return **D** Average Respiratory Quotient during the first 2h after food return for sham-operated rats ad libitum fed (white columns), sham-operated BWm rats (grey columns) and RYGB rats (black columns). As two-way ANOVA revealed a significant F ratio, a Bonferroni post hoc test was used to determine differences between groups (*** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$). Data are shown as mean values ± SEM.

Figure 7: A Mean 24h Energy Expenditure **B** Average Energy Expenditure during the first hour after Ex-4 and saline injection, respectively (1400-1500) **C** Average Energy Expenditure during the dark phase **D** Time course of 24h Energy Expenditure after Exendin-4 (5 µg/kg) and saline s.c. administration. In **A, B** and **C** sham-operated rats ad libitum fed are shown by white columns, sham-operated BWm rats by grey columns and RYGB rats by black columns. In **D** a similar colour code is used with squares representing Exendin-4 and circles representing saline treated rats. As two-way ANOVA revealed a significant F ratio, a Bonferroni post hoc test was used to determine differences between groups (*** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$). Data are shown as mean values ± SEM.

Figure 8: A Average spontaneous food intake during the dark phase **B** Percentage change in food intake after Ex-4 treatment compared to saline treatment **C** Average physical activity during the dark phase **C** Average Respiratory Quotient during the dark phase after Exendin-4 (5 µg/kg) and saline s.c. administration for sham-operated rats ad libitum fed (white columns), sham-operated BWm rats (grey columns) and RYGB rats (black columns). As two-way ANOVA revealed a significant F ratio, a Bonferroni post hoc test was used to determine differences between groups (*** = $p < 0.001$, ** = $p < 0.01$). Data are shown as mean values ± SEM.