Metabolic effects of diets differing in glycaemic index depend on age and endogenous GIP

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Keywords: GIP receptor knockout, glycaemic index, insulin resistance, body fat mass, locomotor activity, hypothalamic satiety factors, aging

Abbreviations: GI, glycaemic index, GIP, glucose-dependent insulinotropic polypeptide; Gipr, GIP receptor; GTT, glucose tolerance test; ITT, insulin tolerance test

Word count: abstract 243, main text 3929

Abstract

Aims/hypothesis: High vs low glycaemic-index (GI) diets unfavourably affect body fat mass and metabolic markers in rodents. Different effects of these diets could be age-dependent, as well as mediated, in part, by carbohydrate-induced stimulation of glucose-dependent insulinotropic polypetide (GIP) signalling. *Methods:* Young-adult (16 weeks) and aged (44 weeks) male wild-type (C57BL/6J) and GIP-receptor knock-out (Gipr-/-) mice were exposed to otherwise identical high-carbohydrate diets only differing in GI (20-26 weeks intervention, n=8-10/group). Diet-induced changes in body fat distribution, liver fat, locomotor activity, markers of insulin sensitivity and substrate oxidation were investigated, as well as changes in the gene expression of anorexigenic and orexigenic hypothalamic factors related to food intake. *Results:* Body weight significantly increased in young-adult high vs low-GI fed mice (two-way ANOVA, $p<0.001$), regardless the Gipr genotype. The high-GI diet in young-adult mice also led to significantly increased fat mass and changes in metabolic markers that indicate reduced insulin sensitivity. Even though body fat mass also slightly increased in high vs low-GI fed aged wild-type mice ($p<0.05$), there were no significant changes in body weight and estimated insulin sensitivity in these animals. However, aged Gipr-/- vs wild-type mice on high-GI diet showed significantly lower cumulative net energy intake, increased locomotor activity, and improved markers of insulin sensitivity. *Conclusions:* The metabolic benefits of a low-GI diet appear to be more pronounced in younger animals, regardless the Gipr genotype. Inactivation of GIP signalling in aged animals on a high-GI diet, however, could be beneficial.

Introduction

High vs low glycaemic-index (GI) diets significantly increase body fat mass and insulin resistance in rodents [1, 2]. In humans, however, beneficial effects of low-GI diets have not been consistently shown [3-7], partly explained by the known problem of controlling confounding factors such as fiber intake and the lack of suitable control groups [8]. Further, in the majority of observational studies that reported associations of GI with risk of type 2 diabetes and cardiovascular disease participants were relatively young [3, 9-11]. However, metabolic responses to nutritional challenges might differ dependent on age. Indeed, the only available long-term prospective cohort study investigating potential effects of diets differing in GI in aged participants (mean age 71 years) showed no associations of the GI both with metabolic markers and risk of coronary heart disease [12]. Notably, also in rodents the reported beneficial effects of low vs high-GI diets are exclusively derived from studies exposing young animals [1, 13-18] or, in the only available long-term study, beginning the dietary intervention in young age [2].

Effects of diets differing in GI could be further mediated, in part, by carbohydrate-induced stimulation of glucose-dependent insulinotropic polypetide (GIP) signalling. GIP is released following fat or carbohydrate consumption and modulates insulin secretion, as well as fat metabolism [19] and locomotor activity [20]. We have recently shown that deficiency of the GIP receptor (Gipr) also affects cumulative food intake, at least in the estrogen deficient state [21]. Consumption of low-GI soluble dietary fiber reduces circulating GIP in diabetic humans [22], probably because of delayed carbohydrate absorption. Further, distinct age-associated changes on body composition and insulin resistance in Gipr knock-out (Gipr-/-) vs wild-type mice fed a normal diet have been recently reported [23].

Therefore, both the age and the Gipr genotype could influence effects of high vs low-GI diets on body composition and metabolic markers. To test these hypotheses, we performed a 20-26

weeks dietary intervention study exposing young-adult (16 weeks) vs aged (44 weeks) male wild-type (C57BL/6J) and Gipr-/- mice to otherwise identical high-carbohydrate diets only differing in GI. Effects of the diets on body composition and markers of insulin sensitivity and substrate oxidation were investigated in all groups of mice. Locomotor activity and expression of hypothalamic factors involved in the regulation of food intake were additionally investigated in aged animals.

Material and Methods

Animals

The protocol for all animal experiments was approved by the local governmental animal ethic review board (Brandenburg, Germany). The animals were kept in accordance with the NIH guidelines for care and use of laboratory animals. Animals were housed individually at a temperature of 22°C with a 12:12-h light-dark cycle in cages with soft wood bedding. The generation of Gipr-/- mice in the C57BL/6 background used in these experiments has been previously described [24].

In the first experiment, 16-week-old male Gipr-/- and control (wild-type) mice were allocated to four groups (n=10 per group) and were fed high vs low-GI diets for 20 weeks, with unlimited access to liquids and experimental diets (Tab 1). Diets were isoenergetic and only differed in starch composition/absorption rate and as such the GI, as detailed below.

In the second experiment, 44-week-old animals were exposed to a similar protocol (n=8 mice per group). However, because after 20 weeks of dietary intervention no significant differences in body weight were seen in aged mice, the dietary intervention in these animals was extended for further 6 weeks, resulting in a 26 weeks dietary intervention period.

After the experimental period animals were sacrificed in fed state. Organs were isolated after rapid preparation. Hypothalamic tissue was submerged in nitrogen and immediately stored at minus 80 °C until further RNA preparation.

Characterisation of experimental diets

Prior to the main experiments, postprandial glucose and insulin responses to the here used experimental high (100% amylopectin, 0% amylose) vs low (30% amylopectin, 70% amylose) GI diets were tested in a separate group of male C57BL/6J mice (n=16). In order to achieve comparable food intake with both diets, eight mice per group were trained for five days. Animals consumed a small portion of standard chow, by introducing a 1g-pellet (Altromin 1324 fortified, Altromin, Lage Germany) into the cage of individually housed mice at 08.00 a.m., following an over-night fast. After 10 min left over pellets were removed. During the training period mice were given free access to standard chow from 11:00 a.m. to 20:00 p.m. On day 5 animals received 500 mg of experimental diets differing only in starch composition. Blood samples from the tail vein (0 min) for measurement of plasma glucose and insulin levels were drawn in the overnight fasted state. Further blood samples were drawn only from mice that consumed the whole portion of the test meals within 5 min (30, 60, 90 and 120 min). Metabolizable macronutrients of diets were calculated according to the energy contents of casein (15.7 kJ/g), carbohydrates (16 kJ/g), and fat (38 kJ/g). The macronutrient composition of the diets is shown in Table 1.

Digestibility of the diets and cumulative energy intake

Feces samples of animals were collected over one week during the third month of dietary intervention. Food intake was recorded for further analysis of energy balance. After drying, energy content of diet samples and feces was determined by bomb calorimetry (IKA C5003, IKA Werke, Germany). Digested energy (defined as diet energy intake (kJ/g) minus energy

loss via the feces (kJ/g) was calculated for all groups of mice. Digestibility of the diet (expressed in percent) was defined as [(digested energy (kJ/g)/diet energy intake (kJ/g)) x 100]. Cumulative digested energy was calculated over an experimental period of 18 weeks in young-adult animals and over 22 weeks in aged animals, respectively, by multiplying the respective measured diet energy intakes with digestibility, which was measured over one week.

Body composition

Body composition (fat mass, lean mass, and free fluids) was measured every two to four weeks using nuclear magnetic resonance spectroscopy (Mini Spect MQ 10 NMR Analyser Bruker, Karlsruhe, Germany). Body weight was recorded every week.

Analysis of hepatic triacylglycerols

Frozen liver tissue was ground in liquid nitrogen to a homogenous powder. 100 mg of tissue was homogenized in 5 ml of 10 mM sodium phosphate buffer containing 1 mM EDTA and 1% polyoxyethylene 10 tridecylethan, using an Ultra-Turrax (IKA Werke, Germany). Samples were centrifuged (10 min, 20000 g) and the supernatant was incubated at 70 °C for 5 min. Triacylglycerols (triglyceride reagent, SIGMA) and protein (DC protein assay, Bio-Rad) levels were analysed in triplicates. Because of potential degeneration processes due to different storage times until measurement in young and aged groups results were normalized to wild-type group with high-GI diet in both experiments.

Response to intraperitoneal (i.p) insulin injection (insulin tolerance test (ITT))

Insulin sensitivity was estimated in fed state after 17 weeks of dietary intervention both in young-adult and aged animals, using intraperitoneal (i.p.) injection of insulin (0.75 IU/kg body mass, Actrapid®, Novonordisk), as described previously [25].

Glucose tolerance test (GTT)

Glucose tolerance tests were performed at week 15 in young-adult mice and at week 20 in aged mice by intraperitoneal glucose injection after an over-night fasting, as detailed previously [25]. Because no significant difference in body weight after 20 weeks of high vs low-GI diets in aged mice was observed, we (i) extended the intervention period in aged mice by a further 6 weeks, and (ii) used a smaller dose of glucose injection (1g/kg body weight instead of 2g/kg body weight) in aged vs young-adult animals in order to further increase the sensitivity of the GTT.

Indirect calorimetry

Oxygen consumption ($V(O_2)$) and CO_2 production ($V(CO_2)$) were assessed by indirect calorimetry in individual mice, using an open respirometric system (gas analyser: Magnos 16 and Uras 14, Hartmann & Braun, Germany). Mice were unrestrained and had free access to their respective high vs low-GI diets. $V(O_2)$ and $V(CO_2)$ production were determined every 6 min over a 22-h period. Respiratory quotient (RQ) was calculated by division of $V(CO_2)$ to $V(O_2)$. Recorded energy expenditure was normalized for metabolic body weight (body weight in $kg^{0.75}$). RQ measurements were performed after 12 weeks of dietary intervention in young-adult mice and after 20 weeks of dietary intervention in aged mice, respectively.

Spontaneous locomotor activity analysis

Mouse locomotor activity within home cage environment was measured in aged mice, using an infrared light system (TSE, Bad Homburg, Germany). The sensors registered the activity of the animal by sensing the body-heat image and its spatial displacement over time. Activity was recorded at week 25 over 24 hours, after 2 days of adaption, and was expressed as counts per hour.

RNA extraction and real time RT-PCR

Total RNA was extracted from total hypothalamus of animals in non fasted state by RNeasy lipid tissue kit® (QIAgen GmbH, Germany). Mouse hypothalamic expression of the mRNA encoding neuropeptide Y (Npy), agouti-related peptide (Agrp), cocaine-amphetamine-related transcript (Cart) and pro-opiomelanocortin (Pomc) were measured using Biosystems 7300 real-time RT-PCR system as described previously [21]. Cycle threshold values from each experimental sample were used calculating the respective gene and 18S mRNA as compared to the standard. The oligonucleotide specific primers were:

18S up 5´-CGGCTACCACATCCAAGGAA-3´, lo5´-GCTGGAATTACCGCGGCT-3´
probe: 5´-GACGGCAAGTCTGGTGCCAGCA-3´
Npy up5'-ACAGAAAACGCCCCCAGAAC-3', lo5'-CGGGAGAACAAGTTTCATTTCC-3'
probe 5'AGGCTTGAAGACCCTTCCATGTGGTGAT-3'
AgRP up5'-ACAACTGCAGACCGAGCAGAA-3', lo5'-CGACGCGGAGAACGAGACT-3'
probe 5'-CAGAAGGCAGAAGCTTTGGCGGAGGT-3'
Cart up5'-CGCATTCCGATCTACGAGAAGAA-3', lo5'-CCTGGCCCCTTTCCTCACT-3'
probe: 5'-CCAAGTCCCCATGTGTGACGCTGGAG-3'
Pomc up5'-GAGAGGCCACTGAACATCTTTGTC-3', lo5'-
TGCAGAGGCAAACAAGATTGG-3', probe 5'-AGAGAGCTGCCTTTCCGCGACAGG-3'

Statistical analysis

Quantitative date are presented as means±SE. Data were analysed using two-way analysis of variance (ANOVA) to detect influences of the diet, the genotype, and the combined effect of diet and genotype (diet x genotype). Two-tailed Student`s t test for unpaired samples was used for subgroup analyses. Time course longitudinal changes of body-weight were analysed using repeated-measured ANOVA with body-weight at specific time points given as within subject

factor, and the diet and the genotype given as between-subject factors. Area under the curve (AUC) calculation was performed by the trapezoidal method. Insulin resistance index was estimated by homeostasis model assessment insulin resistance (HOMA-IR). All data were calculated using SPSS 14, Chicago, USA. A p-value <0.05 was considered significant.

Results

GI of the test meals

After the training period six of eight animals in each group consumed the entire test meals. Differences for glucose and insulin at single time points are shown in Fig. 1a and 1b.

Body weight gain only in younger adult mice under high vs low-GI diets

Body weight at baseline was significantly lower in the young-adult vs aged mouse cohorts (p<0.001), as expected. The influence of the diet on the time course of increases in body weight was significant in young-adult (p<0.001) (Fig. 2a), but not in aged mice (p=0.12) (Fig. 2b). Both in young and aged animals the Gipr-/- genotype had no significant effect on changes in body weight (p>0.55). When comparing the 20-week intervention period of young-adult mice with the first 20 weeks period in aged animals the interaction of time x diet x age showed a significant influence (p<0.001). However, differences in body weight between groups in aged mice did not reach significance level, although we extended the intervention period in aged animals by further 6 weeks (26 vs 20 weeks, as compared to the younger mouse cohort). Therefore, the significant influence of the high-GI diet on body weight appeared to be age-dependent.

Increased body fat and hepatic triacylglycerol content under high-GI diet

The diet significantly influenced changes in body fat both in young-adult mice after 9 and 16 weeks (two-way ANOVA, $p<0.001$) (Fig. 2c) and in aged mice after 8 and 24 weeks ($p<0.05$) (Fig. 2e) of dietary intervention. However, in subgroup analyses significant differences in body fat between groups were only detected in young-adult mice (wild-type, high vs low-GI diet, after 9 and 16 weeks $p<0.01$, Gipr-/-, high vs low-GI diet, after 9 and 16 weeks $p<0.05$), but not in aged mice (Fig. 2e, $p>0.05$, respectively). There was no effect of the Gipr genotype on body fat in both in young-adult and in aged mice ($p=0.69$ and $p=0.26$, respectively). Relative changes in body fat showed similar results, with a significant effect of the diet at week 16 in young-adult ($p<0.001$) and week 24 in aged ($p=0.013$) mice. Two-way ANOVA analysis of lean mass alteration in young wild-type mice under high and low-GI and Gipr-/- under high and low-GI diets (21.1±0.3, 21.0±0.3, 21.2±0.3 and 21.1±0.3 g) was neither influenced by the genotype ($p=0.81$), nor by diet ($p=0.92$) and diet x genotype interaction ($p=0.98$) after 16 weeks. There was also no influence of these three factors in aged mice after 24 weeks (21.5±0.5, 20.8±0.2, 20.6±0.3 and 21.5±0.4, with $p=0.81$, $p=0.731$ and $p=0.08$, respectively).

In agreement with in vivo body fat NMR analysis, hepatic triacylglycerol content was significantly increased both in young (Fig. 2d, $p<0.001$) and aged animals (Fig. 2f, $p=0.02$) fed the high-GI diet, with no influence of the Gipr-/-genotype ($p>0.46$).

Higher energy digestion with high vs low-GI diets, regardless age and Gipr genotype

Digestibility of diets (%) was significantly elevated in high vs low-GI fed wild-type animals, both in younger (95.3±0.2 vs. 90.8±0.4, $p<0.001$) and aged (94.7±0.3 vs. 89.5±0.9, $p<0.001$) mice. Similar differences were observed in younger (95.2±0.3 vs. 90.1±0.4, $p<0.001$) and aged (94.8±0.6 vs. 90.8±0.5, $p<0.001$) Gipr-/- mice. There was no difference in energy digestion between younger and aged mice.

Gipr-/- prevents an increase in cumulative energy intake under high-GI diet in aged animals

In young-adult mice, cumulative net energy intake measured by recording of food intake over 18 weeks was not significantly different between groups, regardless the diet and the Gipr genotype (Fig. 2g). In aged mice, however, cumulative net energy intake was significantly influenced by the diet after 11 (p=0.006) and 22 weeks (p=0.015), respectively, with a significant interaction of genotype x diet (p=0.031) after 22 weeks, indicating an increased cumulative net energy intake under high-GI only in wild-type aged mice (Fig. 2h).

Improved estimated insulin sensitivity in aged Gipr-/- mice under high-GI diet

The response to i.p insulin injection was tested in all groups of mice after 17 weeks of dietary intervention. No differences in estimated insulin sensitivity were observed in young-adult mice using this method (Fig. 3a), despite the observed diet-induced significant differences in body fat. In aged animals, there was also no difference between Gipr genotypes under low-GI diet (Fig. 3b). However, the observed significant influence of the genotype (p=0.014) and the interaction of genotype x diet (p=0.0019) in $AUC_{glucose}$ analysis (plasma glucose suppression to i.p. insulin injection) indicated a protective effect of Gipr deficiency in aged animals under a high-GI diet.

Two-way ANOVA analysis of changes in HOMA-IR in wild-type mice under high and low-GI (7.2±2.0 vs 5.0±1.4) and in Gipr-/- mice under high and low-GI diets (3.0±0.8 vs 3.5±0.6) supported a significant influence of the genotype (p=0.035) in aged but not in young-adult animals (6.9±1.3 vs 4.7±1.7, and 3.5±1.0 vs 3.5±0.8, p=0.1), whereas diet and the interaction of diet x genotype did not reach significant level, neither in young-adult (p>0.39) nor in aged mice (p>0.29).

Intraperitoneal (i.p.) glucose tolerance tests (GTTs) were additionally performed and used as a further marker for the investigation of insulin resistance. While glucose excursions in young mice were mainly influenced by the high-GI diet (p=0.004), independent of the Gipr-/-

genotype, in aged mice there was a significantly reduced $AUC_{glucose}$ in high-GI fed Gipr-/- vs wild-type animals (p=0.003). These findings were in accordance with the results received after i.p. insulin injection, although the interaction of the Gipr genotype with the diet failed to reach the level of significance (p=0.26). $AUC_{insulin}$ following i.p. glucose injection was not different under high vs low-GI diet in all groups of mice (data not shown).

Improved carbohydrate oxidation in aged Gipr-/- mice under high-GI diet

Results from indirect calorimetry further indicated an influence of dietary GI on metabolism in aged Gipr-/- animals. The RQ in aged high vs low-GI fed Gipr-/- mice was significantly higher during the dark phase where most of dietary intake takes place (Fig. 3f), indicating improved carbohydrate metabolism. This result was in agreement with the observed shift to improved insulin sensitivity. In contrast, the mirrored pattern of RQ as observed in younger Gipr-/- mice suggested an improved rather than deteriorated carbohydrate metabolism under low-GI diet (Fig. 3e). Mean RQ showed no significant differences in high vs low-GI fed wild-type animals, neither in young-adult (p=0.35) nor in aged (p=0.37) mice (data not shown). These data indicated that, in the Gipr deficient state, the age of the animals contributed to diverse outcomes when assessing potential beneficial effects of low vs high-GI diets.

Mean energy expenditure per metabolic mass in young wild-type mice under high and low-GI and Gipr-/- under high and low-GI diets (750.8±0.4, 703.6±18.2 and 735.9±11.2 , 716.2±22.5 $kJ \cdot d^{-1}kg^{-0.75}$) was not influenced by genotype (p=0.95), diet (p=0.08) or diet x genotype interaction (p=0.47) in two-way ANOVA analysis. There was also no influence of these three factors in aged animals (574.3±12.3, 583.9±24.7 and 592.0±16.0, 602.5±9.5 $kJ \cdot d^{-1}kg^{-0.75}$, with p=0.279, p=0.54 and p=0.98 respectively).

Increased locomotor activity in aged Gipr-/- mice under high-GI diet

Given that a significant difference in estimated insulin sensitivity was detected in aged high-GI fed Gipr-/- vs wild-type mice despite unaltered body weight after 24 weeks of dietary intervention, we hypothesized that potential changes in locomotor activity could have contributed to the observed findings. Therefore, spontaneous locomotor activity was recorded in aged mice after 24 weeks of dietary intervention (Fig. 4).

Spontaneous locomotor activity during the dark phase, where most of the natural activity takes place in rodents, showed a significant influence of the genotype (two-way ANOVA, $p<0.05$) and a trend in interaction of diet x genotype ($p=0.09$), indicating increased activity levels in the high-GI fed Gipr-/- vs wild-type animals. No differences between groups were observed during the light phase (Fig. 4).

Changes in hypothalamic orexigenic and anorexigenic factors in aged mice

Two-way ANOVA analysis of mRNA expression of hypothalamic factors related to energy intake revealed a significant genotype x diet interaction in aged mice for Agrp transcription ($p=0.022$), and an influence of the diet on Pomc transcription ($p<0.046$). Npy and Cart were not significantly influenced by both factors (Fig. 5). Unexpectedly, the anorexigenic factors Pomc and Cart in both high-GI fed wild-type and Gipr-/- animals even tended to increase, likely representing a compensatory mechanism. Orexigenic Agrp tended to decrease only in Gipr-/- mice under low-GI diet (subgroup analysis, Fig. 5), while Npy showed no obvious tendencies between groups.

Discussion

Nutrition strongly modulates risk factors in the development of obesity and metabolic disorders [26]. High vs low-GI diets increase body fat mass and insulin resistance in rodents [1, 2, 13-17], and have also been proposed to be potentially harmful in humans [27, 28].

Nevertheless there are numerous pre-clinical reports assessing the effect of diets on body weight, benefits of low-GI diets have not been consistently shown in clinical studies [6, 29-33]. We hypothesized that controversial results from human studies might be explained, in part, by differences in age of the investigated subjects, as indicated by findings from prospective cohort studies [3, 12]. Further, apart from dietary fat, dietary carbohydrates are the main physiological stimulators of GIP secretion [34, 35]. Therefore, diets varying in carbohydrate absorption and as such GI could have diverse effects in wild-type vs Gipr deficient states, thereby modulating insulin secretion, fat metabolism, and locomotor activity [19, 20, 34, 35].

Herein, we present novel findings showing that a high vs low-GI diet significantly increased body weight in young-adult, but not in aged mice, regardless the Gipr genotype. Increased fat mass in younger wild-type animals on long term high vs low-GI diets was further observed, which is in agreement with a recently published study [2], reporting significantly increased body fat in 129ScPas mice fed a high-GI diet from young age and with most of the diet induced differences in body fat being apparent as early as 10-12 weeks after the intervention had started. Importantly, when beginning the high-GI intervention in aged animals, as performed in the present study, no diet-induced differences on body weight were observed. Further, diet-induced differences on fat mass were less pronounced in aged animals, even after extending the dietary intervention by further 6 weeks. These data indicate that low vs high-GI diets appeared to have more pronounced effects on body weight and fat mass in young-adult vs aged animals in the present study.

Results from glucose tolerance tests indicated that carbohydrate metabolism in young-adult animals under high GI diet was indeed deteriorated. These changes were independent of the Gipr genotype in young-adult animals, whereas the Gipr genotype appeared to be the driving factor for alterations in carbohydrate metabolism in high-GI fed aged mice. Interestingly, aged high-GI fed Gipr-/- showed significantly improved insulin sensitivity, which was likely

caused by the combination of lower cumulative net energy intake and significantly increased locomotor activity.

To our knowledge, this is the first report showing the effect of a high-GI diet on the expression of hypothalamic neuropeptides. Previous reports have suggested that the rapid absorption of glucose after consumption of high-GI meals induces a sequence of hormonal and metabolic changes that promote excessive food intake in obese subjects [36]. The increased levels of the orexigenic neuropeptide Agrp in the Gipr-/- animals under high vs low-GI diet may explain an acute stimulation of food intake. However, the total cumulative food intake was unchanged between Gipr-/- mice, probably explained by the compensatory effects of up-regulated anorexigenic Pomc expression. Unexpectedly, the expression of the studied neuropeptides did not significantly change between wild-type and Gipr-/- mice fed with high-GI diet, even though the cumulative food intake was higher in wild-type mice. Therefore, analysis of expression of hypothalamic factors in specific hypothalamic nuclei, as well as the investigation of food reward mechanisms could be useful in future studies.

The exact mechanisms of how chronic consumption of high-GI diets leads to fat accumulation are still under debate. One important factor might be the high-GI diet induced postprandial hyperinsulinemia. Further, in former studies in rats enhanced insulin-stimulated glucose oxidation and glucose incorporation into total lipids with increased fat pads was observed [14]. Therefore, diet-induced increases of insulin levels might alter nutritional partitioning in favour of fat deposition, e.g. by shunting metabolic fuels from oxidation in muscle to storage in fat [36]. Moreover, enhanced de novo lipogenesis that also leads to an increased hepatic triacylglycerol accumulation directly inhibits fatty acid oxidation [37, 38]. This ongoing circle may induce an increase in body weight and fat accumulation in the long-term, although less pronounced detrimental effects in aged animals remain to be explained.

Age-associated accumulation of visceral fat is strongly associated with metabolic disturbances including insulin resistance in humans [23, 39, 40]. In order to recommend the most suitable

and effective diets to our patients, it appears to be important investigating whether age-dependent differences of low vs high-GI diets also exist in humans. Considering the reported smaller effects of diets differing in GI in patients of advanced age [12], well controlled intervention studies in aged humans are recommended to justify the considerable efforts of complying with a low-GI diet. The present study was based on a high carbohydrate low fat diet, as usually performed in rodent GI studies [18]. However, a typical Western style diet is also high in dietary fat. Therefore, as also indicated by recent results from others [18], varying the carbohydrate vs fat content in future clinical studies investigating effects of high vs low-GI diets appears to be relevant.

In conclusion, low vs high-GI diets beneficially affected body weight and metabolic markers in younger mice, regardless the Gipr genotype, whereas no obvious advantage of a low-GI diet could be observed in aged wild-type animals. Blockade of GIP action in aged animals on a high-GI diet, however, significantly improved estimates of insulin sensitivity, indicating that selective pharmacological blockage of GIP action in aged humans consuming high-GI diets could be beneficial.

Acknowledgements

We thank S. Richter, K. Weinert, C. Plaue, and K. Wagner for excellent technical assistance.

Duality of interest

All authors confirmed that there were no dualities of interest. FI and AFHP were supported by a grant of the Deutsche Forschungs Gesellschaft (DFG, PF 164/14-1). MOW was supported by a grant of the German Ministry of Education and Science (BMBF, 0313826A).

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Table 1

Macronutrient composition of experimental diets

	High-GI	Low-GI
Diet (g/kg)		
Casein[a]	200	200
Starch[b] 1: 100 % amylopectin	542	
Starch[b] 2: 70 % amylose 30 % amylopectin		542
Sucrose[c]	85	85
Gelatine[d]	20	20
Soybean oil[e]	50	50
Wheat bran[f]	33	33
Mineral mixture[g]	50	50
Vitamin mixture[h]	20	20
Macronutrient, metabolizable energy (%)		
Protein	23	23
Carbohydrates	65	65
Fats	12	12
Measured Diet energy content (kJ/g)	17.4	17.5

[a] Dauermilchwerk Peiting GmbH, Landshut Germany

[b] National Starch and Chemical GmbH, Hamburg, Germany

[c] EUCO GmbH, Hamburg, Germany

[d] Gelita Deutschland GmbH, Eberbach, Germany

[e] Vandemoortele Deutschland GmbH, Dresden, Germany

[f] SchapfenMühle GmbH, Ulm, Germany

[g] Mineral Mix per 100 g diet: Ca, 930 mg; P, 730 mg; Mg, 80 mg; Na, 440mg; K, 710 mg; S, 170 mg; Cl, 360 mg; Fe, 20 mg; Mn, 10 mg; Zn, 3 mg; Cu, 800 mg; J, 40 mg; F, 400 mg; Se, 20 mg; Co, 10 mg (Altromin GmbH, Lage, Germany)

[h] Vitamin mix containing 17.5 g/100 g DL-methionine; vitamin content in 100 g diet: A, 0.45 mg; D_3, 1.3 mg; K_3, 1 mg; B_1, 2 mg; B_2, 2 mg; B_6, 1.5 mg; B_{12}, 3 mg; niacin, 5 mg; pantothenate, 5 mg; folic acid, 1 mg; biotin, 20 mg; choline chloride, 100 mg; *p*-aminobenzoic acid, 10 mg; inositol, 10 mg; E, 16,4 mg (Altromin GmbH, Lage, Germany)

Figure legends

Figure 1: Characterisation of the experimental diets

Plasma glucose **(a)** and insulin levels **(b)** after ingestion of 500 mg of experimental diets with high-GI (open circles) and low-GI (filled circles). * indicates *p*-value < 0.05.

Figure 2: Body composition and energy intake

There was a significant interaction of diet x time on body-weight in young-adult (two-way ANOVA, $p<0.001$) **(a)**, but not in aged mice **(**$p=0.12$) **(b)**. There was a significant effect of the diet on fat mass in young animals after 9 and 16 weeks ($p<0.001$**) (c)**, which was also significant ($p<0.05$), but attenuated in aged animals after 8 and 24 weeks **(e)**. **)**. Diet-induced changes in hepatic triacylglycerol contents are shown in young mice **(d)** ($p<0.001$) and in aged mice **(f)** ($p=0.02$) (data were normalized to the wild-type group with high-GI diet and set as 1.0).

Cumulative energy intake was comparable in young animals **(g)**, but significantly influenced by the diet in aged animals **(h)** ($p< 0.01$ and $p<0.05$, respectively, after 11 and 22 weeks of dietary intervention), with a significant diet x genotype interaction after 22 weeks ($p<0.05$). White bars: wild-type, high-GI; grey bars: wild-type, low-GI; white hatched bars: Gipr-/-, high-GI; grey hatched bars: Gipr-/-, low-GI. White circles: wild type, high-GI; black circles: wild-type, low-GI; white triangles: Gipr-/-, high-GI; black triangles: Gipr-/-, low-GI, (n=8 – 10 per group).

* indicates *p*-value<0.05, ** indicates *p*-value<0.01 in t-test subgroup analyses.

Figure 3: Markers of insulin sensitivity and substrate oxidation

Plasma glucose levels after i.p. insulin injection after 17 weeks of dietary intervention in young-adult **(a)** and aged **(b)** mice. There was a significant influence of the genotype (two-way ANOVA, $p<0.05$) and a genotype x diet interaction ($p<0.05$) in aged, but not in young mice. AUC is presented in the inserts: white bars: wild-type, high-GI; grey bars: wild-type, low-GI; white hatched bars: Gipr-/-, high-GI; grey hatched bars: Gipr-/-, low-GI.
Glucose levels after i.p. glucose injection after 15 weeks of intervention in young **(c)** and after 20 weeks in aged mice **(d)**. There was a significant influence of diet only in young mice ($p<0.05$), whereas in aged mice a significant influence of the genotype was detected ($p<0.01$). Figures **(e)** and **(f)** show respiratory quotient (RQ) during the dark phase in young-adult (after 12 weeks of dietary intervention) and aged Gipr-/- (after 20 weeks of dietary intervention) mice under high vs low-GI diets. RQ in aged mice was significantly increased **(f)**, indicating improved carbohydrate metabolism under high-GI diet. A mirrored pattern of RQ was observed in younger animals **(e)**. White circles: wild type, high-GI; black circles: wild-type, low-GI; white triangles: Gipr-/-, high-GI; black triangles: Gipr-/-, low-GI). * indicates *p*-value<0.05, ** indicates *p*-value<0.01 in t-test subgroup analyses

Figure 4: Spontaneous locomotor activity

Spontaneous locomotor activity in aged mice, measured during the light phase and the dark phase over a 24 hours period, after 25 week of intervention with high vs low-GI diets. There was a significant influence of the genotype (two-way ANOVA, p<0.05).White bars: wild-type, high-GI; grey bars: wild-type, low-GI; white hatched bars: Gipr-/-, high-GI; grey hatched bars: Gipr-/-, low-GI. * indicates *p*-value < 0.05 in t-test subgroup analyses.

Figure 5: Changes in orexigenic and anorexigenic hypothalamic factors

Real-time RT-PCR analysis of orexigenic and anorexigenic factors in hypothalamus of aged mice. Results were normalized to internal control, and the wild-type group with high-GI diet was set as 1.0. There was a significant genotype x diet interaction on changes in agouti-related peptide (Agrp) transcription (two-way ANOVA, $p<0.05$), and a diet effect on pro-opiomelanocortin (Pomc) transcription ($p<0.05$). Neuropeptide Y (Npy) and cocaine-amphetamine-related transcript (Cart) were unaffected by both factors. White bars: wild-type, high-GI; grey bars: wild-type, low-GI; white hatched bars: Gipr-/-, high-GI; grey hatched bars: Gipr-/-, low-GI.

* indicates *p*-value <0.05, ** indicates *p*-value <0.01 in t-test subgroup analysis

Figure 1

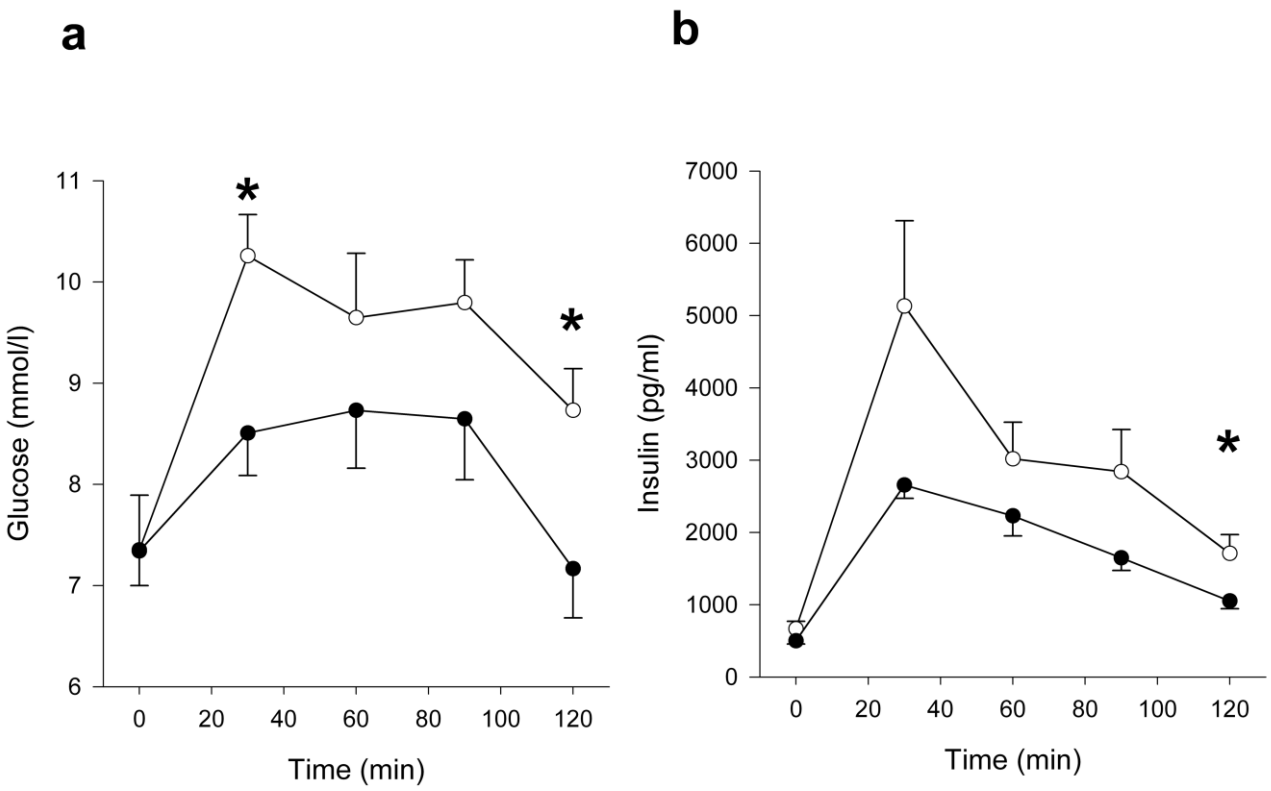


Figure 2

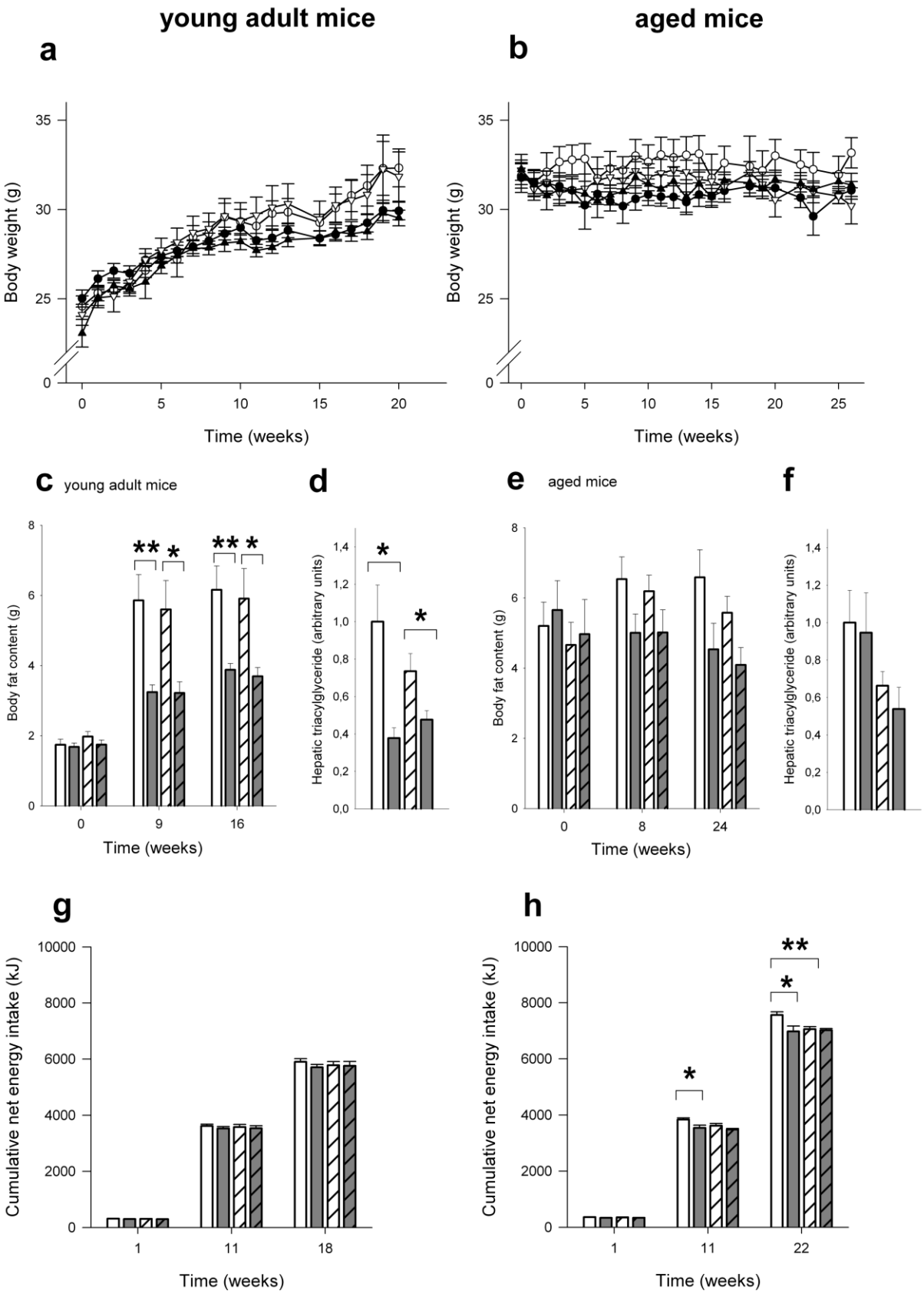

young adult mice
aged mice
a
b
Body weight (g)
Time (weeks)
c young adult mice
d
e aged mice
f
Body fat content (g)
Hepatic triacylglyceride (arbitrary units)
g
h
Cumulative net energy intake (kJ)

Figure 3

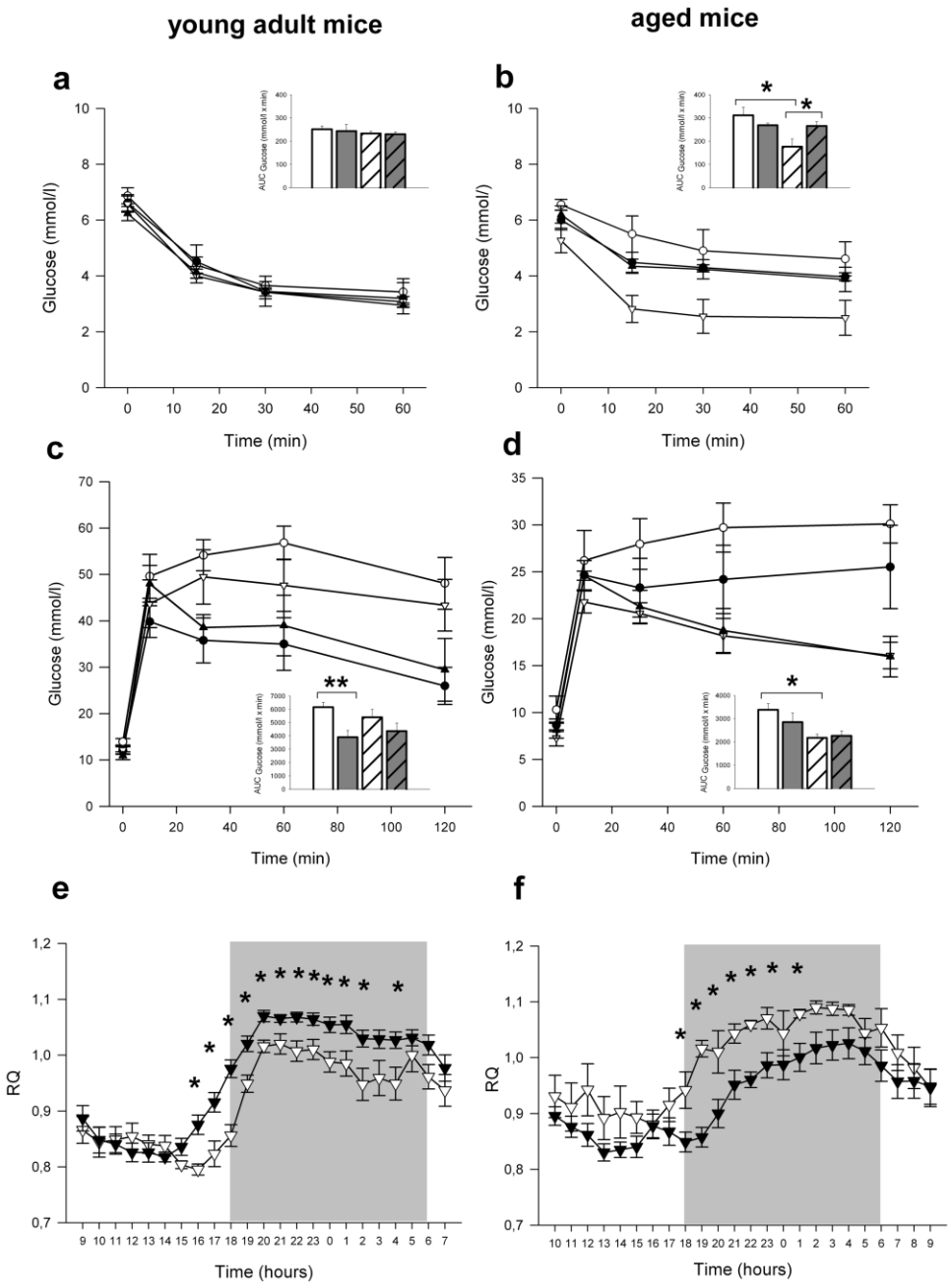

young adult mice
aged mice
a
b
c
d
e
f
Glucose (mmol/l)
Time (min)
AUC Glucose (mmol/l x min)
RQ
Time (hours)

Figure 4

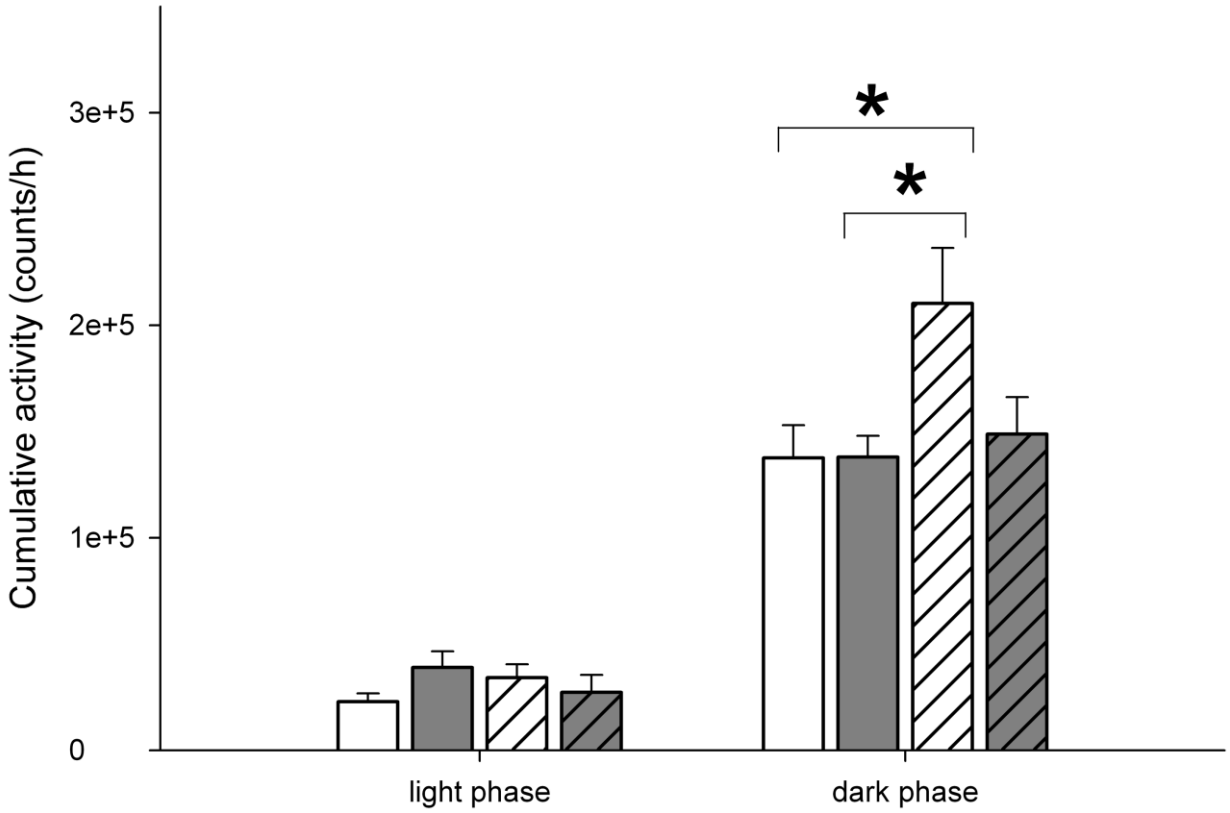
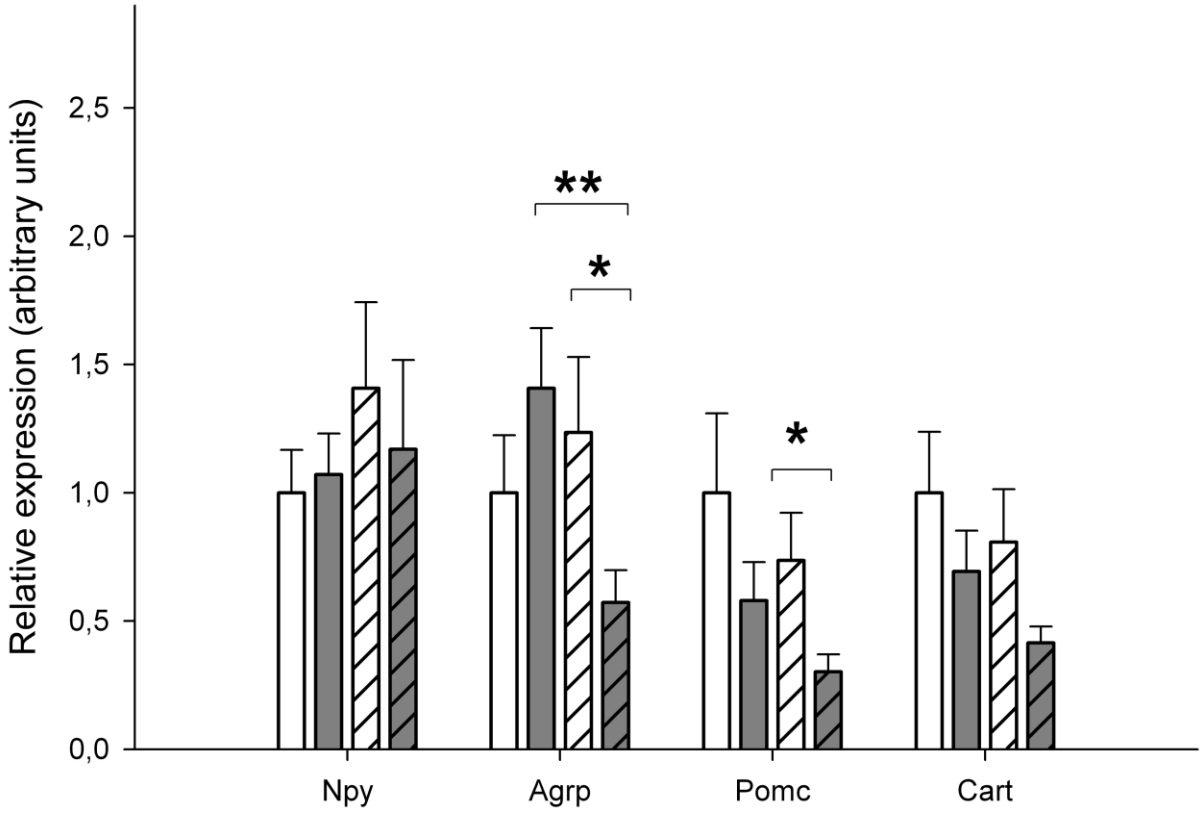

Cumulative activity (counts/h)
3e+5
2e+5
1e+5
0
*
*
light phase
dark phase

Figure 5


Relative expression (arbitrary units)
2,5
2,0
1,5
1,0
0,5
0,0
**
*
*
Npy
Agrp
Pomc
Cart