The effects of queen mandibular pheromone on nurse-aged honey bee (*Apis mellifera*) hypopharyngeal gland size and lipid metabolism

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Abstract

Queen honey bees (*Apis mellifera*) release Queen Mandibular Pheromone (QMP) to regulate traits in the caste of female helpers called workers. QMP signals the queen's presence and suppresses worker reproduction. In the absence of reproduction, young workers take care of the queen and her larvae (nurse tasks), while older workers forage. In nurses, QMP increases lipid stores in abdominal fat tissue (fat body) and protein content in hypopharyngeal glands (HPG). HPG are worker-specific head glands that can synthesize royal jelly used in colony nourishment. Larger HPG signifies ability to secrete royal jelly, while shrunken glands characterize foragers that do not make jelly. While it is known that QMP increases abdominal lipid stores, the mechanism is unclear: Does QMP make workers consume more pollen which provides lipids, or does QMP increase lipogenic capacity? Here, we measure abdominal lipogenic capacity as fatty acid synthase (FAS) activity while monitoring abdominal protein content and HPG size in caged workers. Cages allow us to rigorously control worker age, pheromone exposure, and diet. In our 2-factorial design, 3- vs. 8-day-old workers (age factor) were exposed to synthetic QMP or not (pheromone factor) while consuming a lipid deficient diet. We found that QMP did not influence abdominal FAS activity or protein content, but QMP still increased HPG size in the absence of dietary lipids. Our data revealed a positive correlation between abdominal protein content and HPG size. Our findings show that QMP is not a strong modulator of lipogenic capacity in caged worker bees. However, our data may reflect that QMP mobilizes abdominal protein for production of jelly, in line with previous findings on effects of honey bee Brood Pheromone. Overall, our study expands the understanding of how QMP can affect honey bee workers. Such insights are important beyond regulatory biology, as QMP is used in various aspects of beekeeping.

1. Introduction

Pheromones are chemical signals used for communication between members of the same species [1]. In insects, some of the extensively studied roles of pheromones include attraction of mates, signaling of reproductive status, maintenance of social hierarchy, and recognition of kin [1]. Insect pheromones are categorized as releaser and/or primer pheromones. Releasers elicit immediate behavioral responses within seconds or minutes, while primers produce long-term endocrine or reproductive changes that may take days to operate in full effect [2, 3]. The long-term physiological effects that primer pheromones exhibit largely influence insect colony organization, caste structure, and the division of labor [4]. One of the most intricate and highly studied insect pheromonal systems is that of honey bees (*A. mellifera*), with approximately 50 substances that have a biologically relevant role in colony life [5].

Each honey bee colony consists of a reproductive queen, non-reproductive females known as workers, and males known as drones [6]. Adult worker honey bees show a division of labor called age polyethism, in which individuals perform different social tasks as they age. Young workers (first 2-3 weeks after emerging from pupation) work inside the nest to groom and nourish all colony members, while older workers venture outside to collect pollen, nectar, water and propolis [7, 8]. This age polyethism is accompanied by physiological specializations, such as the young workers (nurses) having larger abdominal lipid stores and hypopharyngeal glands (HPGs) to support their role in colony nourishment, while foragers are leaner and rely on sugars to fuel their flights [7, 8, 9].

Queen mandibular pheromone (QMP), released by the queen bee, influences worker division of labor and physiology [4]. QMP signals the presence of a queen and is categorized as

both a releaser and a primer. As a releaser, QMP prompts young worker bees to groom the queen through a retinue response and further spread the pheromone in the colony [3, 5]. As a primer, QMP suppresses genes associated with foraging while activating genes associated with nursing [10]. These are a few examples of the well-known functions of QMP. Past studies show that QMP can increase abdominal lipid stores and the size of HPGs in nurse-aged workers [11, 12, 13, 14]. HPGs are paired head glands that can produce royal jelly [4]. They contain a higher concentration of lipids than other tissues in the head, and bees fed diets high in lipids develop larger HPGs [15, 16]. In some research on QMP, however, the diet of workers contained pollen that provides both lipids and amino acids [15, 16]. This natural food composition makes it difficult to tease apart how QMP acts to influence physiology. Are the changes in lipid stores and HPG size a simple function of increased pollen consumption, just due to the lipid fraction of the diet, or potentially also relying on lipogenic capacity? These are some of many possibilities.

To assess a possible increase in lipogenic capacity in the fat body of workers exposed to QMP, we measured the activity of fatty acid synthase (FAS), an enzyme that catalyzes a rate-limiting step of *de novo* lipogenic capacity that combines malonyl-CoA with acetyl-CoA to produce long-chain fatty acids, thus serving as a measure of lipogenic capacity [17]. These long-chain fatty acids are stored as triglycerides in honey bees and most other animals [18]. Thus, in this study we use a FAS activity assay to test whether QMP influences lipogenic capacity in nurse-aged worker bees. We fed the bees a lipid-deficient diet to eliminate the confounding aspects of their natural, pollen-containing food on HPG size. We hypothesized that QMP increases lipogenic capacity in nurses' abdominal fat bodies (functionally homologous to liver and white adipose tissue), resulting in larger lipid stores and HPGs.

Our specific design was a 2 factorial cage experiment with an age factor and a pheromone factor: 3-day-old (representing developing bees not yet physiologically competent to become nurses or foragers) or 8-day-old (representing bees at peak nursing age) were exposed to synthetic QMP, or not. FAS activity in the fat body was normalized by measuring the amount of abdominal protein per nurse-aged bee. We monitored HPG size by measuring the area of the glands' acini. For each cage, we also monitored the depletion of the lipid-deficient diet as a function of worker mass, to control for whether QMP influenced food consumption.

2. Materials and Methods

2.1 Honey bees

The experiments were performed in September through November of 2023 at Arizona State University Campus, Tempe. Three frames from 3 different hives were collected, sealed in a mesh cage, placed in an incubator at 33 °C, and kept humidified using an open dish of water at the bottom of the incubator. After 24 h, newly emerged bees were collected and placed in small (5 × 12 × 16 cm) Plexiglass and mesh cages (30 bees/cage). Bees were fed 30% w/v sucrose solution *ad libitum* in 20 ml syringes with their tips cut off and placed at the top of the cage as described previously [19]. To feed the bees sufficient protein for development of HPGs without feeding them lipids, we also fed the bees artificial diets using soy as a protein source as described previously [20]. A paste containing 20% total protein was made with 21.47% soy protein isolate (MP Biomedical) and 78.26% honey and fed to the bees using 1.5 mL Eppendorf feeders placed at the bottom of each cage (Fig. 1). Consumption of sucrose solution and protein paste was calculated by weighing the feeders every 24 hours.

Fig 1. Cage design used for the queen mandibular pheromone treatments. The dimensions measured were 90 mm × 120 nm × 158 mm. Because approximately half the cage was used, the effective depth was 55 mm.

A 2 × 2 factorial experimental design was used, and each cage was assigned to an age-group (3 and 8-day-old bees) and a treatment group of QMP+ or QMP−. One cage was constructed for each combination of age and presence/absence of QMP and replicated 3 times, yielding a total of 12 cages (1 cage × 4 treatment groups × 3 replicates; Fig. 2). To produce bees of specific ages, cages were set up either 3 or 8 days prior to each sampling day. The cages were maintained at 33 °C in a dark, humidified incubator and mortality was recorded every day in each cage. Cages also received the QMP treatment for the duration of those 3 or 8 days. Synthetic QMP ("TempQueen"; Betterbee Inc.) was presented as a slow-release strip placed at the bottom of the QMP+ cages, while QMP− cages did not receive a QMP strip, as in an earlier study [21]. The strip was used according to the manufacture instructions and contained an equivalent of the 5-component blend of QMP. It mimics exposure to natural QMP, a mixture of 9-keto-2-(E)-decenoic acid, the enantiomers of 9-hydroxy-2-(E)-decenoic acid (88% R-(–) and 12% S-(+)), methyl p-hydroxybenzoate, and 4-hydroxy-3-methoxyphenylethanol [2]. QMP is not volatile as it is spread by honey bee workers via trophallaxis, antennation, and cuticular contact [21]. Thus, all cages were kept in the same incubator. During sampling, bees were anesthetized on ice, euthanized, and dissected for subsequent analyses.

Fig 2. Pictorial depiction of the 2-factorial design used in the study. The 2-factorial design that represented all combinations of the presence vs absence of synthetic queen mandibular pheromone ('QMP') and 3 vs 8-day-old nurse-aged honey bees ('age'). This resulted in 4 experimental treatments, each applied to one cage of bees. The design was replicated three times,

resulting in three replicates. The small boxes labelled inside each large box represent the cage identity (12 total cages used).

2.2 Fatty acid synthase (FAS) activity measurements

Fat body fatty acid synthase (FAS) activity was measured using a previously described method with minor modifications [22]. Briefly, the abdominal carcass (complete abdominal cuticle with adhering fat body tissue minus the stinger, ovaries, gut, and crop) was isolated from workers, pooled in pairs of two, homogenized in phosphate-buffered saline containing protease inhibitors (11697498001; Roche Applied Science; Indianapolis; IN; USA), sonicated for 30 seconds, and centrifuged at 10,000 × *g* for 5 minutes at 4°C. The resulting supernatant was collected and assayed immediately in a 96-well microplate. In each well, 33.3 µL of supernatant was mixed with 163.3 µL of 2.0 M potassium phosphate buffer, pH 7.1, 16.7 µl of 20 mM dithiothreitol, 20 µl of 0.25 mM acetyl-CoA, 16.7 µl of 60 mM EDTA. To initiate the reaction, 33.3 µl of 0.39 mM malonyl-CoA was added to each well. FAS activity was measured as the oxidation of NADPH at 340 nm and 37 °C using a UV/VIS spectrophotometer (Synergy H1 Multimode Reader, BioTek). A background correction was made for the oxidation of NADPH in the absence of malonyl-CoA. Background and sample wells were both measured in duplicate. This assay was replicated 3 times with a total of 144 abdomens sampled. FAS activity was calculated as nmol of NADPH oxidized/min/abdomen. Additionally, FAS activity was normalized by the amount of total soluble protein in each sample, measured with a BCA Assay Kit (Thermo Scientific) according to manufacturer's instructions, giving FAS activity in nmol/min/mg.

2.3 Hypopharyngeal gland measurements

To determine whether QMP affects HPG acini size, honey bee heads from the same bees used for FAS activity were flash frozen in liquid nitrogen and kept in a -80 °C freezer until they were dissected. 18 heads per treatment group were dissected, resulting in a total of 72 heads. For each head, the HPGs were first dissected into a glass plate with concave deep wells containing 40 µl of 10x Giemsa for 7 min. They were then transferred into a flat microscope slide that contained 60 µl of 1x PBS buffer (37 mM NaCl, 2.7 mM KCl, and 10 mM PO4, pH 7.4) and visualized at 60 to 80x magnification, as previously described [23]. The glands were visualized under a Leica M205C stereoscope with a Leica DFC450 camera using the Leica Applications Suite v4.5 software. A blind observer was then told to select 10 acini per bee with the criteria that the acini were in focus, had clear attachment points to the collecting duct, and were average relative to all the acini in the photo. The area (mm) of those 10 selected acini per bee was measured using ImageJ by the researcher. The areas were then averaged per bee and analyzed as a pooled sample in which the heads that were pooled together corresponded to the abdomens that were pooled together during the FAS analysis.

2.4 Statistical analysis

The effects of age and QMP on abdominal FAS activity, abdominal protein, and HPG acini area were preferentially processed with an ANOVA test using three variables: age, treatment, and replicate. Datasets that were analyzed with ANOVA adhered to its assumptions of normality, estimated by normal probability plots of the datasets and a Shapiro-Wilks test, and homogeneity of variances, determined by a Levene's test. The minimum p value for a significant dataset was 0.05. When assumptions were not met, in the case of normalized FAS activity dataset and protein/sucrose consumption, a non-parametric test was used, specifically, a Kruskal-

Wallis test. The non-parametric test was followed by a Dunn's Post hoc test if needed. Replicates were included in the ANOVA to control for differences between replicates. Three replicates were performed for the study and each replicate reflects a combination of cage/day and FAS assay plate variation. The relationships between HPG acini size and abdominal protein and abdominal FAS activity were analyzed using linear regression. Because the data deviated significantly from the assumptions of normality, the relationship between abdominal protein and FAS activity was analyzed using a Kendall-Theil Sen Siegel non-parametric linear regression. All statistics were analyzed with R version 4.3.1, Rstudio, and the packages 'FSA', 'performance', 'mblm', and 'rcompanion' [24, 25, 26, 27, 28]. The sample size used for measuring protein paste and sucrose consumption was $n = 3$. The sample size for measuring abdominal FAS activity, normalized FAS activity, abdominal protein, and HPG acini area was $n = 18$ pooled samples per treatment group. The sample size for the regression analyses was $n = 35$, except for the case of abdominal protein and abdominal FAS which was $n = 72$.

3. Results

3.1 Food Consumption

Protein paste consumption did not significantly differ between any of the four treatment groups (Kruskal-Wallis, chi-squared = 2.6923, df = 3, $P = 0.4415$; Fig 3). Furthermore, sucrose consumption did not significantly differ between any of the four treatment groups (Kruskal-Wallis, chi-squared = 0.63041, df = 3, $P = 0.8894$; Fig 4). Protein consumption did not differ significantly between replicates (chi-squared = 3.7308, df = 2, $P = 0.1548$) but sucrose consumption did differ significantly between replicates (chi-squared = 7.4974, df = 2, $P = 0.02355$).

Fig 3. The relationship between age/QMP and protein consumption in mg/bee/day. A non-parametric Kruskal-Wallis test showed that there were no statistical differences between each treatment's protein consumption ($P = 0.4415$). Each point represents the average protein paste consumption for one cage ($n = 3$ cages). The boxplot shows the mean (middle black line of box), the interquartile range (box boundaries), and the minimum and maximum values of the distribution. Outliers are points outside the maximum and minimum of the distribution.

Fig 4. The relationship between age/QMP and sucrose consumption in mg/bee/day. A non-parametric Kruskal-Wallis test showed that there were no statistical differences between each treatment's sucrose solution consumption ($P = 0.8894$). Each point represents the average sucrose consumption for one cage ($n = 3$ cages). The boxplot shows the mean (middle black line of box), the interquartile range (box boundaries), and the minimum and maximum values of the distribution. Outliers are points outside the maximum and minimum of the distribution.

3.2 Abdominal FAS activity and abdominal protein

Age ($F_{1,60} = 2.010$, $P = 0.16140$) and QMP treatment ($F_{1,60} = 0.698$, $P = 0.40668$) had no significant effect on abdominal FAS activity in nmol NADPH oxidized per minute per abdomen (Fig 5). Furthermore, there was no interaction effect between age × QMP treatment ($F_{1,60} = 0.484$, $P = 0.48931$), QMP treatment × replicate ($F_{2,60} = 0.962$, $P = 0.29039$), or age × QMP treatment × replicate ($F_{2,60} = 0.726$, $P = 0.48796$). However, there were significant differences in abdominal FAS activity between the three replicates ($F_{2,60} = 29.323$, $P < 0.001$; S1 Fig) and a significant interaction effect between age × replicate ($F_{2,60} = 5.152$, $P = 0.00862$). To normalize the FAS activity assay, the abdominal protein (in mg) of each pooled sample was measured. Abdominal protein was significantly higher in 8-day-old than 3-day-old bees ($F_{1,60} = 35.693$, $P <$

228 0.001; Fig 6) and significantly different between replicates ($F_{2,60} = 36.000$ $P < 0.001$; S2 Fig); it
229 was not significantly affected by QMP treatment ($F_{1,60} = 0.032$, $P = 0.8596$), age × QMP
230 treatment ($F_{1,60} = 1.057$, $P = 0.3081$), age × replicate ($F_{2,60} = 2.809$, $P = 0.0682$), treatment ×
231 replicate ($F_{2,60} = 0.717$, $P = 0.4925$), or age × treatment × replicate ($F_{2,60} = 1.240$, $P = 0.2968$).

232 **Fig 5. The relationship between age/QMP treatment and abdominal FAS activity (NADPH**
233 **oxidized) in nmol/min.** Age ($F_{1,60} = 2.010$, $P = 0.16140$) and QMP treatment ($F_{1,60} = 0.698$, $P =$
234 0.40668) had no significant effect on abdominal FAS activity in nmol NADPH oxidized per
235 minute per abdomen. Furthermore, there was no interaction effect between age × QMP treatment
236 ($F_{1,60} = 0.484$, $P = 0.48931$), QMP treatment × replicate ($F_{2,60} = 0.962$, $P = 0.29039$), or age ×
237 QMP treatment × replicate ($F_{2,60} = 0.726$, $P = 0.48796$). Each point represents the average
238 abdominal FAS activity for each pooled sample of two bees ($n = 18$ pooled samples). The
239 boxplot shows the mean (middle black line of box), the interquartile range (box boundaries), and
240 the minimum and maximum values of the distribution. Outliers are points outside the maximum
241 and minimum of the distribution.

Fig 6. The effect of age/QMP treatment and abdominal protein quantity in mg. Abdominal protein was significantly higher in 8-day-old than 3-day-old bees ($F_{1,60} = 35.693$, $P < 0.001$). It was not significantly affected by QMP treatment ($F_{1,60} = 0.032$, $P = 0.8596$), age × QMP treatment ($F_{1,60} = 1.057$, $P = 0.3081$), age × replicate ($F_{2,60} = 2.809$, $P = 0.0682$), treatment × replicate ($F_{2,60} = 0.717$, $P = 0.4925$), or age × treatment × replicate ($F_{2,60} = 1.240$, $P = 0.2968$). Each point represents the average abdominal protein for each pooled sample of two bees ($n = 18$ pooled samples). The boxplot shows the mean (middle black line of box), the interquartile range (box boundaries), and the minimum and maximum values of the distribution. Outliers are points outside the maximum and minimum of the distribution.

3.3 FAS activity normalized to abdominal protein

Using the abdominal protein quantity, FAS activity was normalized per pooled sample in nmol of NADPH oxidized per min per mg of protein. Normalized FAS activity significantly differed between the three replicates (chi-squared = 23.87, df = 2, $P < 0.001$; S3 Fig) and the four treatment groups (Kruskal-Wallis, chi-squared = 9.3498, df = 3, $P = 0.02498$; Fig 7). Comparisons between the four treatment groups showed that only the 3-day-old QMP + and 8-day-old QMP – groups differed significantly from each other (Dunn's *post-hoc* test, *Padj* = 0.0397).

Fig 7. The effect of age/QMP treatment and normalized FAS activity in nmol/min/mg. Normalized FAS activity significantly differed between the four treatment groups (Kruskal-Wallis, chi-squared = 9.3498, df = 3, $P = 0.02498$). Comparisons between the four treatment groups showed that only the 3-day-old QMP + and 8-day-old QMP – groups differed significantly from each other (Dunn's *post-hoc* test, $Padj = 0.0397$). No other treatment groups were significantly different from one another. Each point represents the average normalized FAS activity for each pooled sample of two bees ($n = 18$ pooled samples). The boxplot shows the mean (middle black line of box), the interquartile range (box boundaries), and the minimum and maximum values of the distribution. Outliers are points outside the maximum and minimum of the distribution.

3.4 Average HPG acini area

Mean HPG acini area was significantly higher in 8-day-old than 3-day-old bees ($F_{1,60} = 27.780$, $P < 0.001$; Fig 8) and in bees treated with QMP ($F_{1,60} = 29.156$, $P < 0.001$) and was significantly different between replicates ($F_{2,60} = 6.853$, $P = 0.00209$; S4 Fig). The interaction effect age × treatment × replicate was significant ($F_{2,60} = 4.457$, $P = 0.01568$); the other interaction effects of age × QMP treatment ($F_{1,60} = 0.568$, $P = 0.45392$), age × replicate ($F_{2,60} = 1.338$, $P = 0.27004$), and treatment × replicate ($F_{2,60} = 2.369$, $P = 0.10226$) did not significantly affect mean HPG acini area.

277 **Fig 8. The effect of age/QMP treatment and average HPG acini area in mm^2.** Mean HPG
278 acini area was significantly higher in 8-day-old than 3-day-old bees ($F_{1,60} = 27.780$, $P < 0.001$)
279 and in bees treated with QMP ($F_{1,60} = 29.156$, $P < 0.001$). The interaction effect age × treatment
280 × replicate was significant ($F_{2,60} = 4.457$, $P = 0.01568$); the other interaction effects of age ×
281 QMP treatment ($F_{1,60} = 0.568$, $P = 0.45392$), age × replicate ($F_{2,60} = 1.338$, $P = 0.27004$), and
282 treatment × replicate ($F_{2,60} = 2.369$, $P = 0.10226$) did not significantly affect mean HPG acini
283 area. Each point represents the average HPG acini area for each pooled sample of two bees (n =
284 18 pooled samples). The boxplot shows the mean (middle black line of box), the interquartile
285 range (box boundaries), and the minimum and maximum values of the distribution. Outliers are
286 points outside the maximum and minimum of the distribution.

287 ## 3.5 Relationship between HPG size and abdominal metrics

288 Mean HPG acini size was significantly positively predicted by abdominal protein
289 quantity ($F_{1,33} = 12.66$, adjusted $R^2 = 0.2382$, $P = 0.00116$; Fig 9) and by abdominal FAS
290 activity ($F_{1,33} = 5.819$, adjusted $R^2 = 0.1181$, $P = 0.02123$; Fig 10) but not by normalized FAS
291 activity ($F_{1,33} = 0.944$, adjusted $R^2 = -0.001558$, $P = 0.3379$). Abdominal FAS activity
292 significantly increased with an increase in abdominal protein quantity (Kendall-Theil Sen Siegel
293 linear regression: estimate = 1.5049 ± 1.4016, $V = 2265$, Efron's pseudo $R^2 = 0.0498$, $P < 0.001$;
294 Fig 11).

295 **Fig 9. Regression scatter plot between abdominal protein (mg) and HPG acini area (mm^2).**
296 Mean HPG acini size was significantly predicted by abdominal protein quantity ($F_{1,33} = 12.66$,
297 adjusted $R^2 = 0.2382$, $P = 0.00116$). Each point represents the correlation between the pooled
298 sample's (2 bees per) abdominal protein and HPG acini area ($n = 35$).

Fig 10. Regression scatter plot between abdominal FAS (nmol NADPH oxidized/min) and HPG acini area (mm^2). Mean HPG acini size was significantly predicted by abdominal FAS activity ($F_{1,33} = 5.819$, adjusted $R^2 = 0.1181$, $P = 0.02123$). Each point represents the correlation between the pooled sample's (2 bees per) abdominal FAS activity and HPG acini area ($n = 35$).

Fig 11. Non-parametric linear regression scatter plot between abdominal protein (mg) and abdominal FAS (nmol oxidized NADPH/min). Abdominal FAS activity was significantly predicted by abdominal protein quantity (Kendall-Theil Sen Siegel linear regression: estimate = 1.5049 ± 1.4016, $V = 2265$, Efron's pseudo $R^2 = 0.0498$, $P < 0.001$). Each point represents the correlation between the pooled sample's (2 bees per) abdominal protein and abdominal FAS activity ($n = 72$).

Table 1: Regression analysis *P* values between all combinations of abdominal protein, abdominal FAS activity and HPG acini area.

	Abdominal protein	Abdominal FAS activity	HPG acini area
Abdominal protein		9.61×10^{-8} ***	0.00116**
Abdominal FAS activity	9.61×10^{-8} ***		0.02123*
HPG acini area	0.00116**	0.02123*	

311 Asterisks represent significant differences (* denotes $P < 0.05$; ** denotes $P < 0.01$, *** denotes
312 $P < 0.001$) for each correlation. The sample size taken for each regression analysis was $N = 35$,
313 except for abdominal protein and abdominal FAS which was $N = 72$. The full regression analysis
314 statistics can be found in S1 File.

315 # 4. Discussion

316 In this study, we explored if QMP can increase fat body lipids and HPG size in nurse-
317 aged worker bees fed a lipid-deficient diet. We show that workers exposed to synthetic QMP
318 have larger HPGs on average, but we did not detect a change in the bees' lipogenic
319 capacity. Although there were significant replicate effects present, these results simply indicate
320 that there is some non-treatment group variation that causes noise in the data. Thus, for this
321 study, replicate effects are irrelevant to the rest of our results.

322 During the experiment, we monitored food consumption to determine whether QMP
323 increases HPG size through affecting worker lipogenic capacity or through simply increasing the
324 workers' food consumption. Overall, QMP did not influence the depletion of sucrose solution or
325 protein paste in our cages. This finding is consistent with a previous study in which the sucrose
326 and pollen consumption of nurse-aged bees did not differ between queenright-like treatments
327 (QMP) and queenless-like treatments (no QMP) [14]. However, other work has shown that QMP
328 exposure can increase food consumption in nurse-aged bees fed rich diets that contained pollen,
329 as well as in nurse-aged bees fed with poor diets consisting of just sucrose solution [13]. Our
330 results may have differed from these latter results because we used synthetic QMP strips while
331 Ament and colleagues [13] used 0.1 equivalents of QMP dissolved with isopropanol and water
332 on a microscope over slip. Furthermore, there may be mixed results on whether QMP affects

food consumption due to the varying sample sizes in all three studies. While our study had a sample size of 3 cages for consumption, Ament and colleagues [13] had a sample size ranging from 6 to 8 cages and Peters and colleagues [14] had a sample size ranging from around 150 to 300 bees, depending on the treatment group. Because our study had the smallest sample size, our data should be interpreted cautiously.

Age and QMP treatment did not significantly affect FAS activity analyzed per bee, and only age significantly affected FAS activity analyzed per mg of extracted protein. This suggests that QMP does not significantly affect the *de novo* synthesis of lipids in nurse-aged honey bee fat bodies, but caged bees can increase their abdominal protein content as they age. In contrast, recent data from our group show that FAS activity does not differ significantly between 3d and 8d bees collected from natural colonies (Sebastian et. al – manuscript in preparation). Our results may have differed due to the environment of both treatments. While the past study experimented on bees within natural hives who were receiving optimal nutrition from being fed by other nurse bees, our study experimented on bees within cages that received less nutrition. Contrary to our findings, it was previously reported that QMP can increase abdominal lipid stores in bees provided pollen-containing diets, as well as diets consisting only of sucrose solution [11, 12, 13]. How can this diversity of results be explained? One possibility is that QMP reduces the activity levels of young workers as shown before [30]. The mechanism underlying the larger lipid stores is then, simply, reduced energy expenditure.

In our experiment, caged 8-day-old worker bees had larger amounts of abdominal protein than 3-day-old bees, while QMP had no effect on this protein level. Protein levels generally increase in young workers after they emerge from pupation [7], suggesting that our caged workers were able to obtain adequate nutrition. More specifically, abdominal protein levels are

356 correlated with the amount of Vitellogenin (Vg) protein in colony-living worker bees [31]. Vg is
357 an important indicator of nutrition and health in honey bees, and the protein influences several
358 aspects of worker physiology and behavior, including the function of the HPGs [31]. We did not
359 measure Vg in our experiment, but the positive correlation we find between total protein levels
360 and HPG size might be influenced by this protein.

361 Our experiment detected a significantly increased HPG acini size in both 3d and 8d
362 nurse-aged bees exposed to QMP. This increase was not associated with measurable changes in
363 food consumption or FAS activity but correlated with the abdominal protein content. One
364 possible explanation for this result is that QMP plays a role in mobilizing the abdominal protein,
365 such as Vg, to the HPGs to increase their size. Furthermore, QMP was also previously shown to
366 suppress levels of circulating juvenile hormone (JH) [4, 32]. JH can suppress Vg and treatment
367 with JH analog will reduce HPG size [33, 34]. Thus, an alternative explanation is that QMP
368 allowed HPGs to develop because JH was suppressed in this treatment group. However, previous
369 research shows that JH is generally controlled at lower levels in cages with 20 workers or more
370 [20], which suggests that this is not the best explanation for the findings.

371 It is known that lipids found within the HPG are essential to produce royal jelly and lipids
372 found within the fat body are important for nursing behavior in general. Our study aimed to
373 determine if these two pathways are connected in the presence of QMP and whether the
374 functioning of one is necessary for the other. Using a lipid-deficient diet to eliminate the
375 possibility of QMP increasing nurse-aged bees' consumption of lipids, we found that QMP
376 significantly increased HPG size without affecting nurse-aged lipogenic capacity, suggesting that
377 the fat body lipids levels are not as important for the development of HPGs compared to
378 abdominal protein levels. Overall, this study emphasized the importance of how pheromonal

379 regulation can influence adaptive physiology and nutrient storage in worker honey bees. This
380 knowledge provides further insight into basic bee biology and to queen pheromone, which is
381 commercially used in apiculture.

382 Supporting Information

383 **S1 Fig. The effect of replicates on abdominal FAS activity measured in**
384 **nmol/min/abdomen.** Abdominal FAS activity significantly differed between the three replicates
385 ($F_{2,60} = 29.323$, $P < 0.001$)

386 **S2 Fig. The effect of replicates on abdominal protein measured in mg.** Abdominal protein
387 significantly differed between the three replicates ($F_{2,60} = 36$, $P < 0.001$).

388 **S3 Fig. The effect of replicates on normalized FAS activity measured in nmol/min/mg.**
389 Normalized FAS activity significantly differed between the three replicates (chi-squared = 23.87,
390 $P < 0.001$, df = 2).

391 **S4 Fig. The effect of replicates on HPG acini area measured in mm².** Mean HPG acini area
392 significantly differed between the three replicates ($F_{2,60} = 6.853$, $P = 0.00209$).

393 **S1 File. The full regression plot analysis statistics between abdominal protein and HPG**
394 **acini area, abdominal FAS and HPG acini area, and abdominal protein and abdominal**
395 **FAS.**

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Figure 1

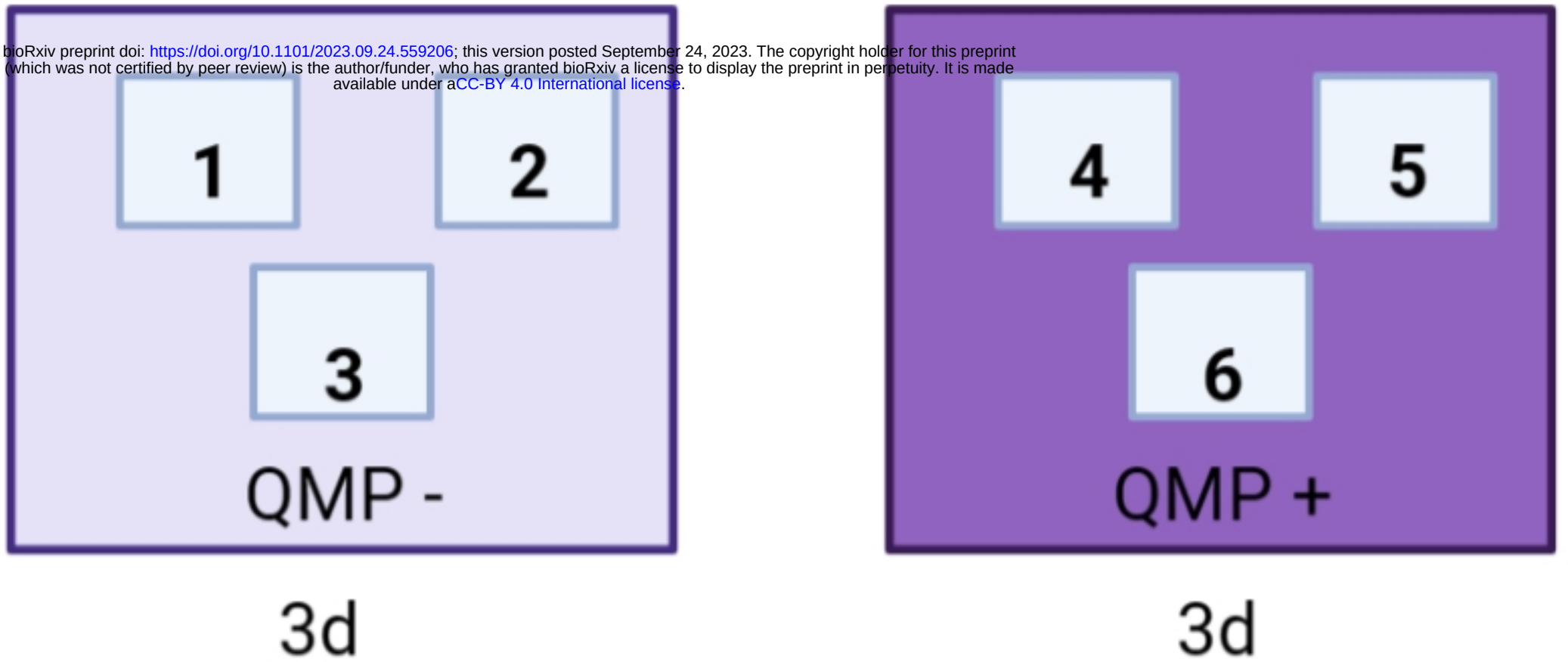

Figure 2

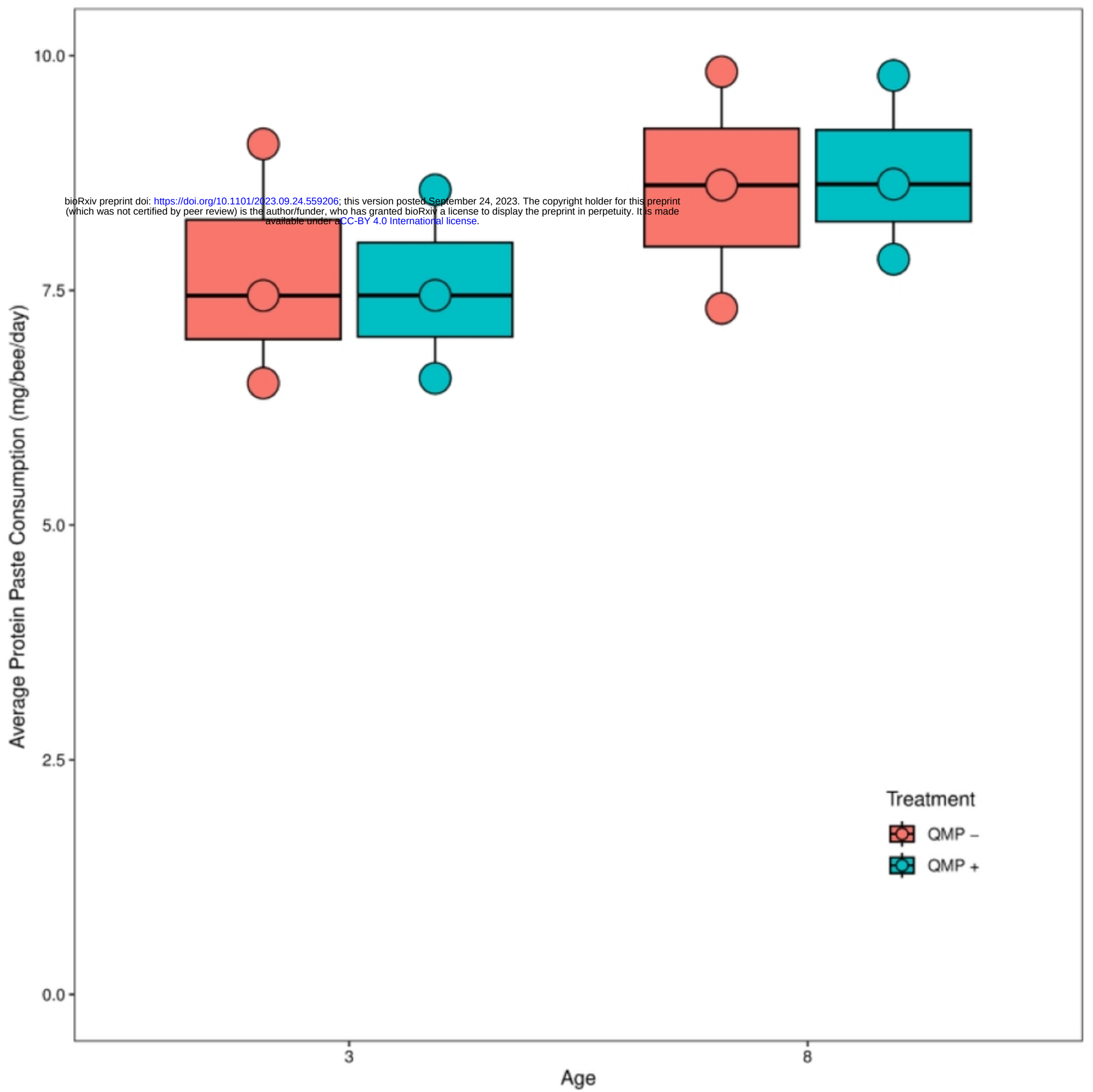

Figure 3

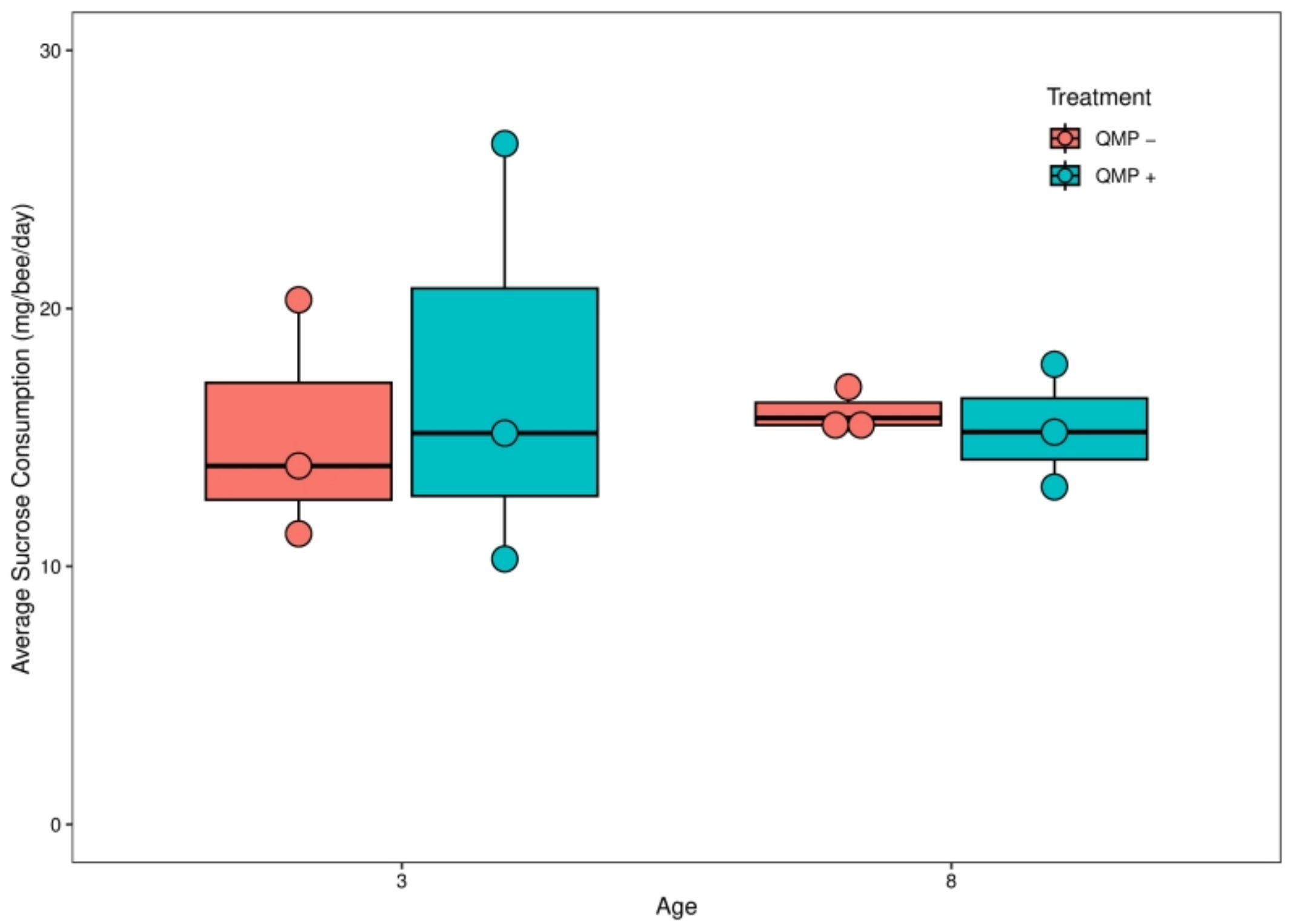
Average Sucrose Consumption (mg/bee/day)
30
20
10
0
3
8
Age
Treatment
QMP –
QMP +

Figure 4

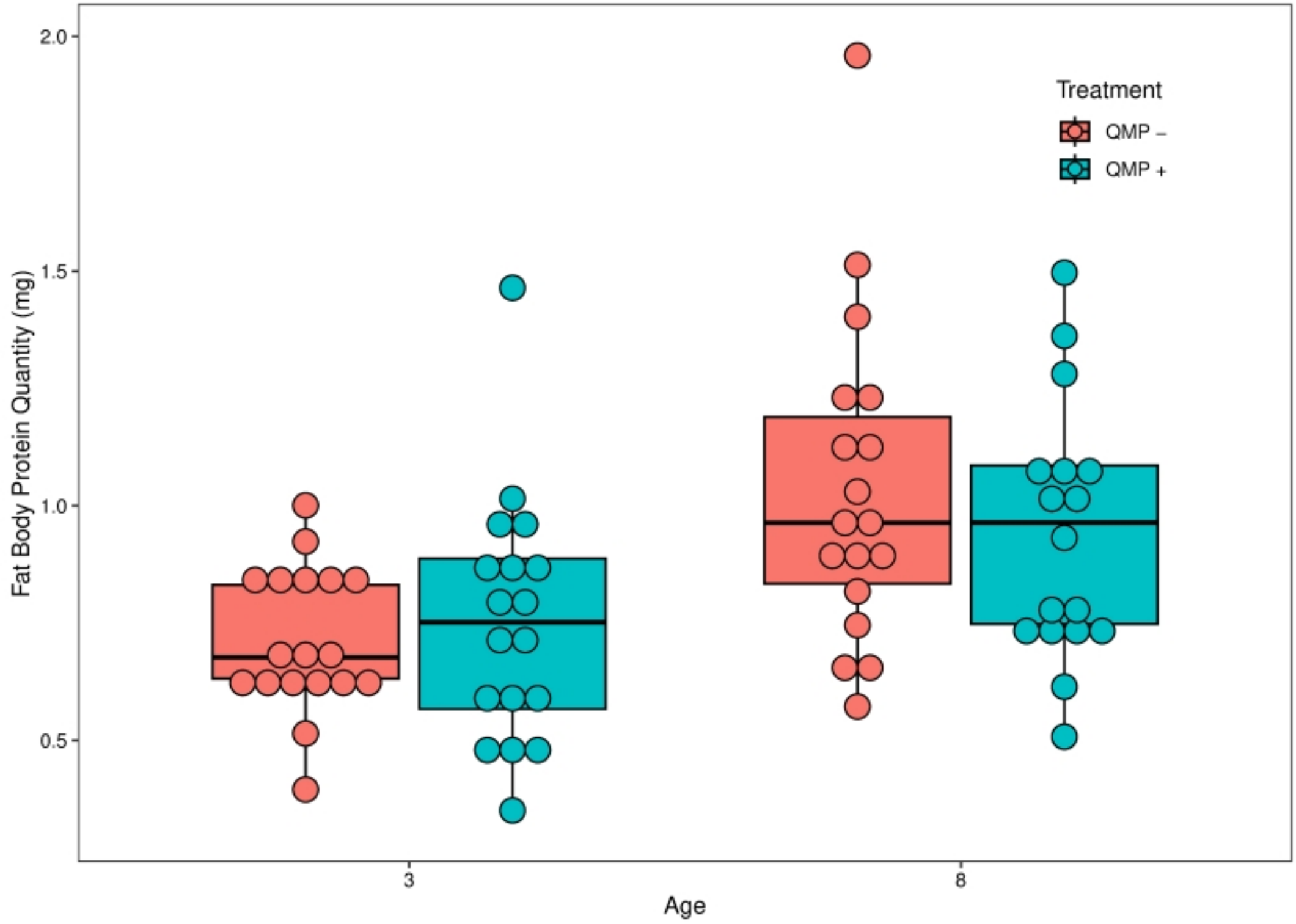
Fat Body Protein Quantity (mg)
2.0
1.5
1.0
0.5
3
8
Age
Treatment
QMP –
QMP +

Figure 6

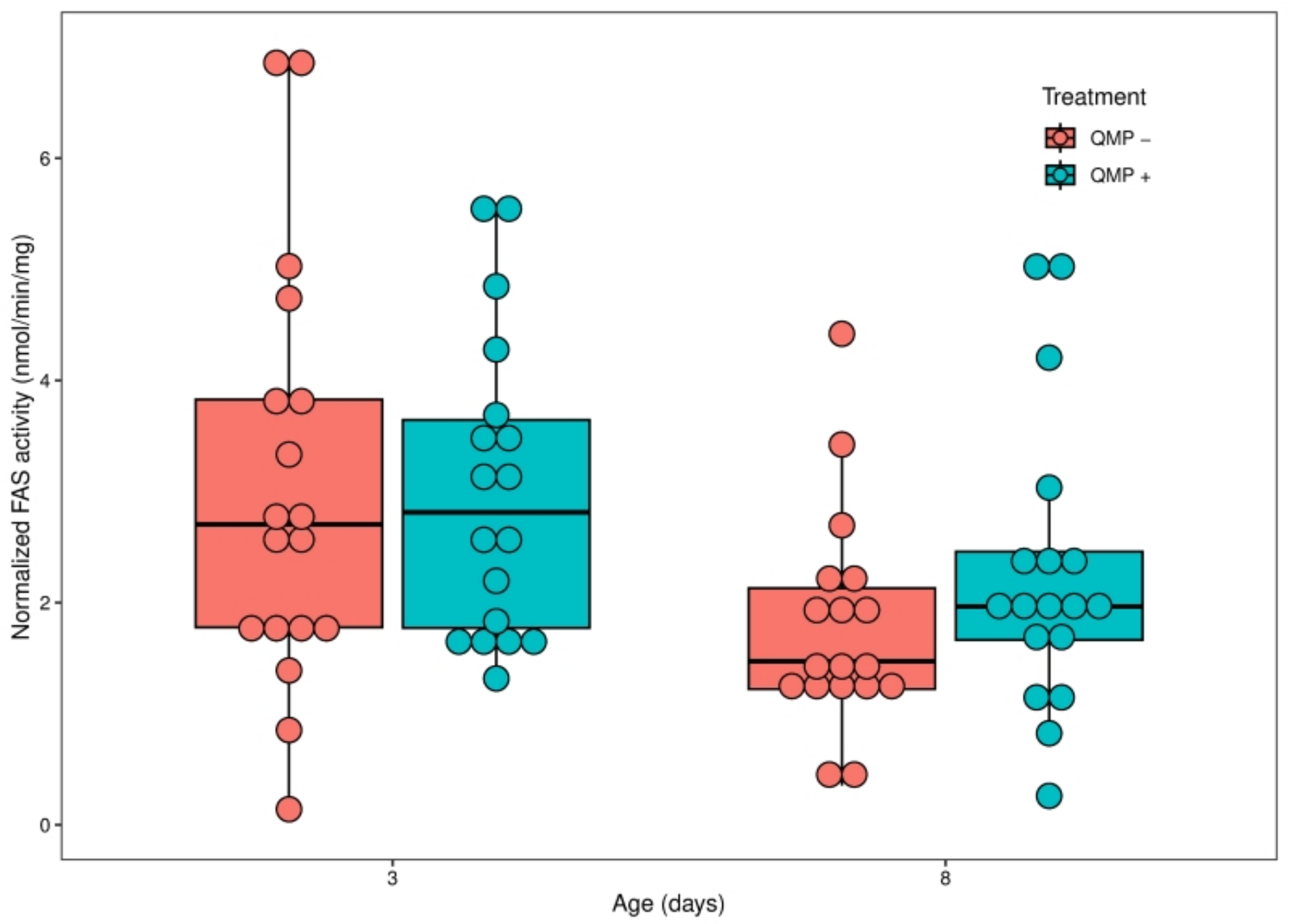
Treatment
QMP –
QMP +
Normalized FAS activity (nmol/min/mg)
0
2
4
6
3
8
Age (days)

Figure 7

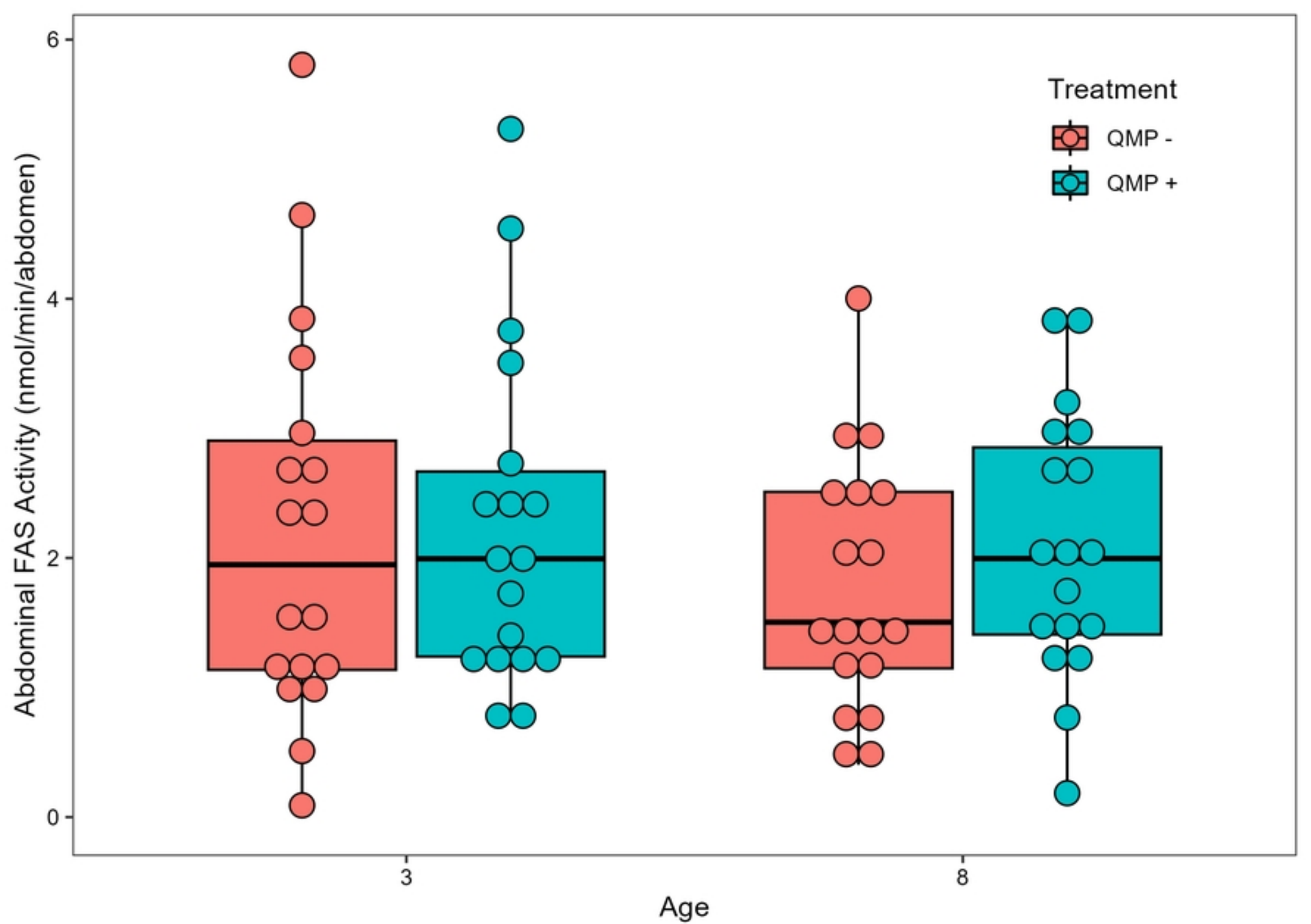
Abdominal FAS Activity (nmol/min/abdomen)
6
4
2
0
3
8
Age
Treatment
QMP -
QMP +

Figure 5

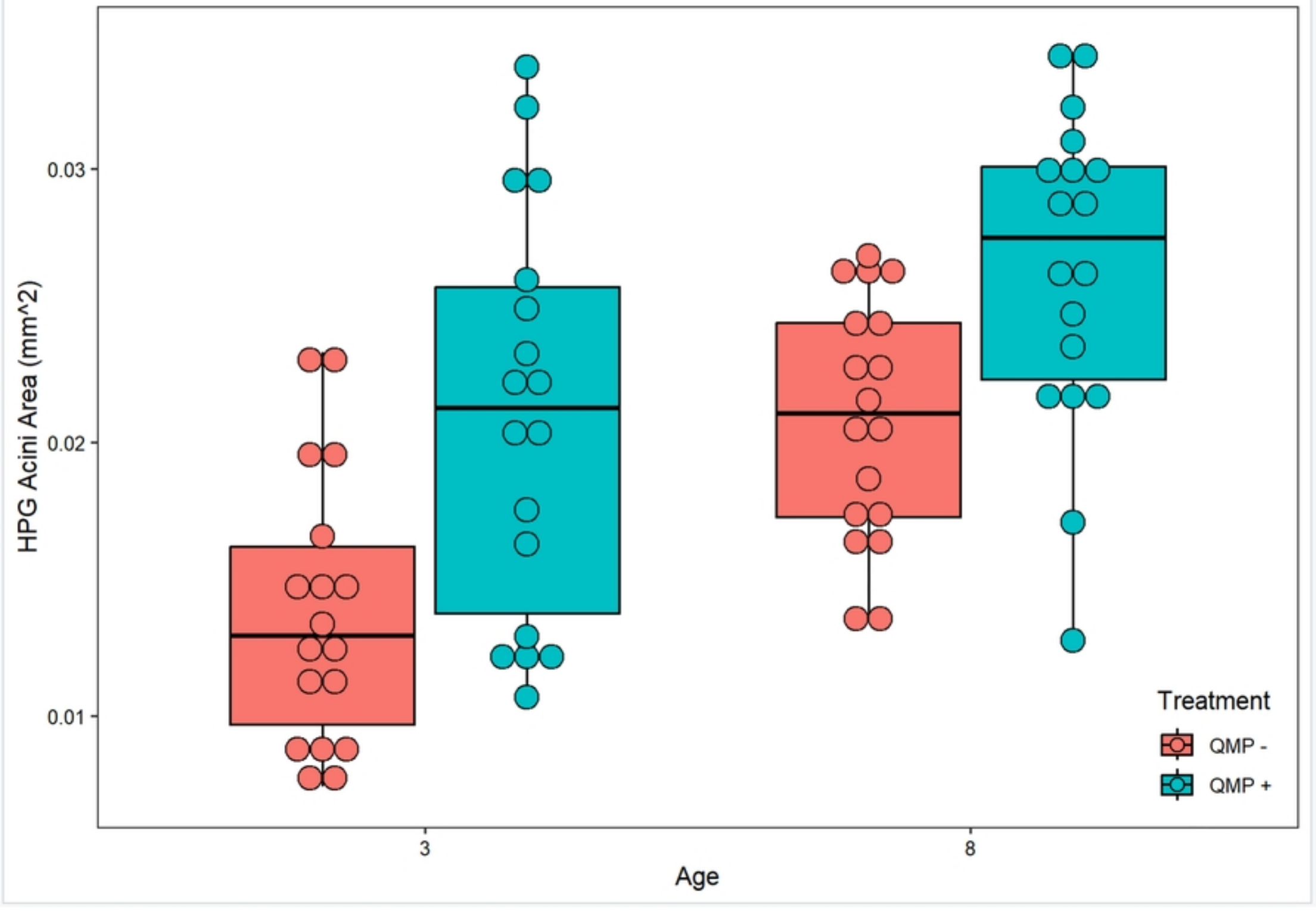

Figure 8

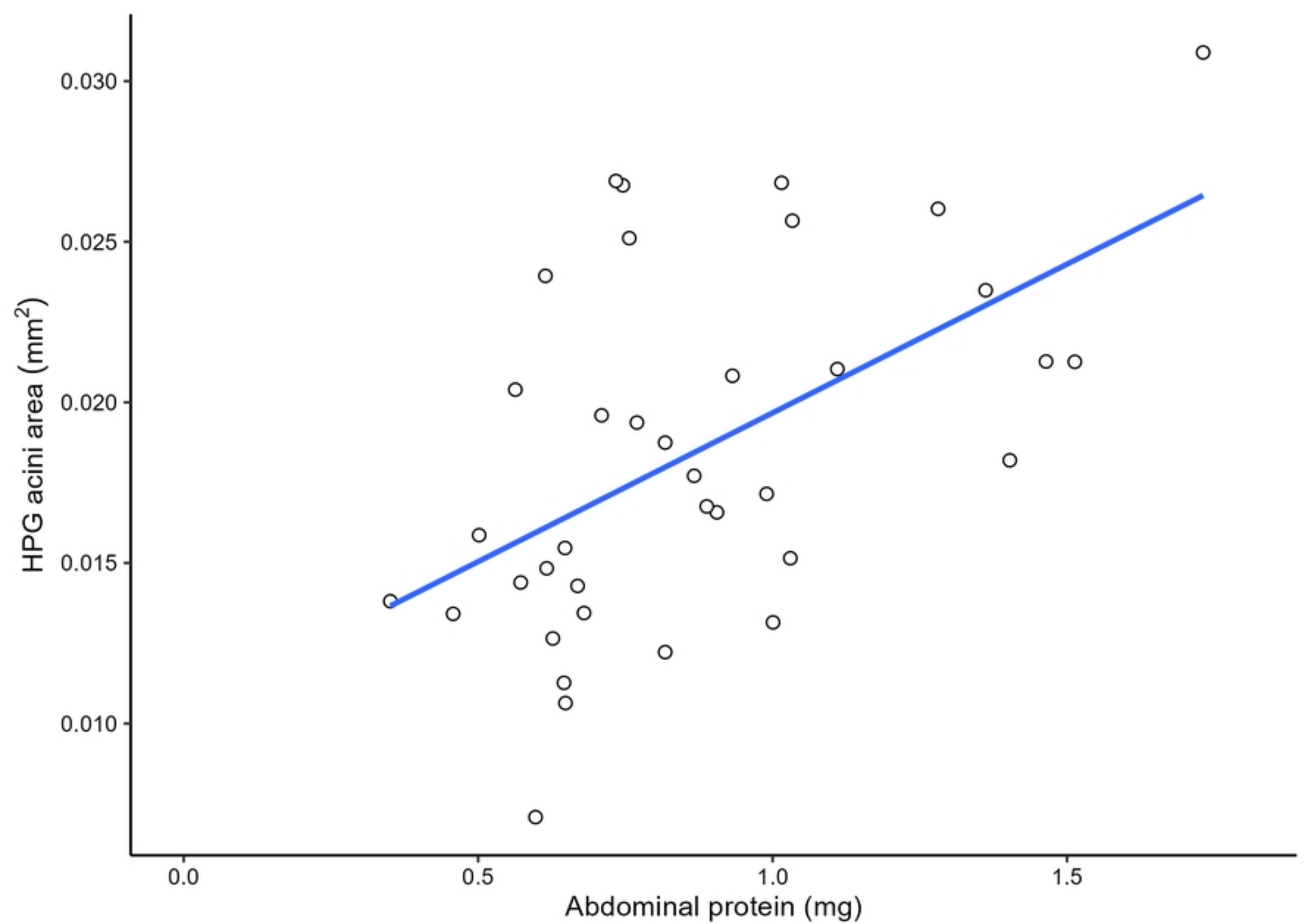
0.030
0.025
0.020
0.015
0.010
HPG acini area (mm^2)
0.0
0.5
1.0
1.5
Abdominal protein (mg)

Figure 9

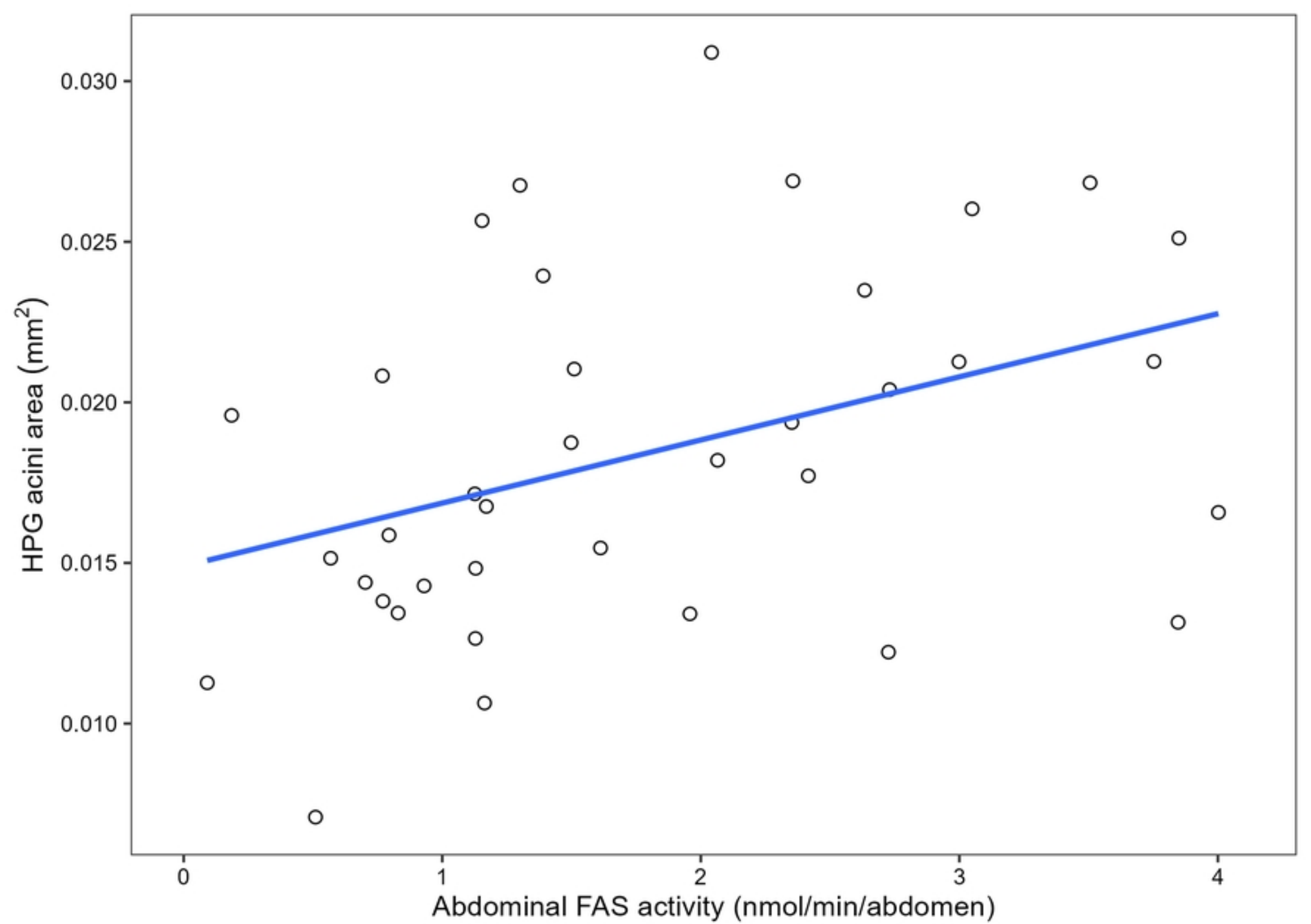
0.030
0.025
0.020
0.015
0.010
HPG acini area (mm2)
0
1
2
3
4
Abdominal FAS activity (nmol/min/abdomen)

Figure 10

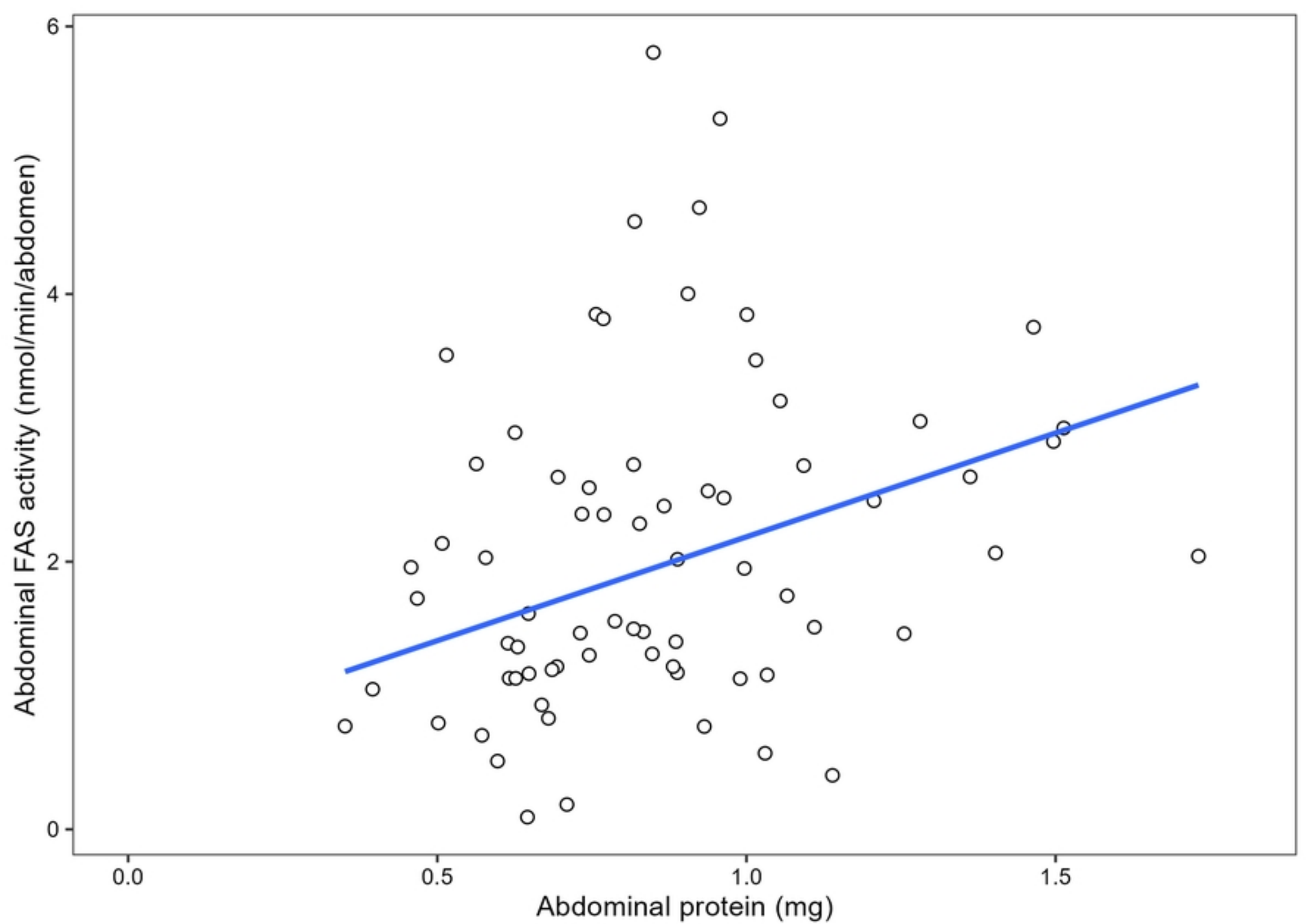
Abdominal FAS activity (nmol/min/abdomen)
6
4
2
0
0.0
0.5
1.0
1.5
Abdominal protein (mg)

Figure 11