Bimatoprost promotes satiety and attenuates body weight in rats fed standard or obesity-promoting diets.

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Abstract

Background

Bimatoprost negatively regulates adipogenesis in vitro and likely participates in a negative feedback loop on anandamide-induced adipogenesis. Here, we investigate the broader metabolic effects of bimatoprost action *in vivo* in rats under both normal state and obesity-inducing conditions.

Methods

Male Sprague Dawley rats were a fed standard chow (SC) diet in conjunction with dermally applied bimatoprost treatment for a period of 9–10 weeks. Body weight gain, energy expenditure, food intake, and hormones associated with satiety were measured. Gastric emptying was also separately evaluated. In obesity-promoting diet studies, rats were fed a cafeteria diet (CAF) and gross weight, fat accumulation in SQ, visceral fat and liver was evaluated together with standard serum chemistry.

Results

Chronic bimatoprost administration attenuated weight gain in rats fed either standard or obesity-promoting diets over a 9–10 weeks. Bimatoprost increased satiety as measured by decreased food intake, gastric emptying and circulating gut hormone levels. Additionally, SQ and visceral fat mass was distinctly affected by treatment. Bimatoprost increased satiety as measured by decreased food intake, gastric emptying and circulating gut hormone levels.

Conclusions

These findings suggest that bimatoprost (and possibly prostamide F2α) regulates energy homeostasis through actions on dietary intake. These actions likely counteract the metabolic actions of anandamide through the endocannabinoid system potentially revealing a new pathway that could be exploited for therapeutic development.

Introduction

In response to changing caloric intake the body can regulate storage capacity together with intrinsic energy expenditure to maintain energy homeostasis (1). Adipose tissue plays a crucial role through its ability to sequester triglycerides and modulate whole body metabolism via adipokine action. However, progressive fat storage within adipose tissue leads to obesity and an elevated risk for the development of adverse health conditions, including type 2 diabetes, elevated serum insulin and lipid imbalance, as well as cardiac, hepatic and pancreatic tissue pathologies (2, 3).

One mechanism linking obesity to these pathogenic features is an inability to effectively sequester triglycerides within adipose tissue (1). Central to this process is adipogenesis which provides the means to expand adipose mass through the recruitment of new adipocytes from pre-adipocyte precursors. A failure of adipogenesis to adequately increase storage capacity in this way leads to increased triglyceride content within existing adipocyte depots. Hypertrophy within the adipocyte can then lead to inefficient triglyceride storage and altered adipokine action, resulting in ectopic triglyceride accumulation within tissues such as the skeletal muscle and liver (3). The biological mechanisms regulating adipogenesis and triglyceride storage by the adipocyte and how these processes can be perturbed in obesity are therefore of great interest.

Within this context, prostamide F2α and its precursor the endocannabinoid, anandamide, have been shown to reciprocally regulate adipogenesis (4, 5). Prostamide-F2α is derived from anandamide by enzymatic conversion through PTGS2 (COX-2) and prostaglandin F synthases (6). Anandamide circulating levels are increased in obesity and promote adipogenesis as well as food intake via CB1 receptors (7). By comparison, prostamide F2α inhibits adipogenesis from both rodent and human progenitors (4, 5) and is likely mediated through prostamide receptor mediated down-regulation of PPARγ and C/EPPα (4). The effects of prostamide-F2α appear targeted on preadipocytes as its potency is reduced by ~ 500-fold on mature adipocytes (6). Consistent with an anti-adipogenic action, bimatoprost also inhibits adipogenesis *in vitro* and its clinical use in glaucoma patients leads to a reduction in peri-ocular fat deposits (4, 8–13). This clinical side effect is consistent with the ability to modulate adipose tissue expansion i*n vivo* at least at the administration site.

These *in vitro* findings present the possibility that these anti-adipogenic effects by bimatoprost might comprise part of a broader prostamide metabolic action i*n vivo*. We therefore investigated the metabolic consequences evoked through chronic administration of bimatoprost, a synthetic analog of prostamide-F2α. In the present study, the broader systemic effects of bimatoprost in rats fed standard or obesity-promoting dietary regimes were investigated through its chronic topical administration to the skin. Body weight, visceral fat, blood chemistry, tissue histomorphology, dietary habits, gastric emptying, and energy consumption and expenditure were monitored. Our findings suggest a novel mechanism by which bimatoprost and perhaps prostamide-F2α more generally is involved in regulating dietary intake and energy expenditure to manage body weight, fat mass, and energy homeostasis.

Materials And Methods

Chemicals.

Bimatoprost was custom synthesized by Torcan Ltd. (Aurora, ON, Canada). Bimatoprost was prepared in 67% ethanol/33% saline.

Food intake and indirect calorimetry

Male Sprague-Dawley rats between 39 and 45 days old and weighing between 200-250g were recruited in a time-staggered fashion and fed a Standard rodent chow (Labdiet, St. Louis, MO 63144, USA) formula for two weeks. At “week 0” rats were randomly allocated to either weight-matched vehicle control (67% ethanol) or 3% bimatoprost-treated groups (30mg/ml; ~10-15mg/kg) respectively daily for 10 weeks. At specified times, indirect calorimetry, food/water intake, feces excretion, and locomotor activity were measured using metabolic cages. Body weight measurements and total food intake were performed weekly for each pair of caged rats calculated as the weight of food lost from the “food hopper”.

Indirect calorimetry was performed using an open-circuit system of the Comprehensive Laboratory Animal Monitoring System (Columbus Instruments, Columbus, OH). Animals were individually acclimated to respirometry chambers for approximately 72 h followed by a 24-h period of data collection. Rats continued to receive daily administration of either vehicle or bimatoprost. For all studies rat pairs were housed in a controlled environment (12-h light, 12-h dark cycle; room temperature, 22 ± 2 ◦C) with ad libitum access to a SC-diet and water. All procedures involving the use of animals were approved by the University of Auckland Animal Ethics Committee.

Effect of bimatoprost-treatment on blood hormone levels

Animals were fed the SC-diet for two weeks as described above. At week 0 rats in control and designated bimatoprost-treated groups were topically administered vehicle (67% ethanol) daily for 3 days prior to fasting overnight for 16 hours starting from late afternoon. For the acute experiment, rats then received either vehicle or one of three bimatoprost doses (3%, 9% or 25%). Tail vein blood was withdrawn at 30 min and 60 min post-administration. For the sub-chronic experiment, rats continued to receive daily administration of the same vehicle or selected bimatoprost dose for a further three days. Rats were then fasted overnight for 16 hrs and blood samples again collected at 30 min and 60 min after the last dose administrated as described above. DPPIV (Millipore, Cat#: MPDPP4) and serine protease (Pefabloc, Roche) inhibitors were added immediately to collected blood samples with sera and subsequently stored at -80◦C. Hormone measurements were performed in duplicate using a rat metabolic hormone magnetic bead panel with analytes described in Table 1 and performed according to the manufacturer instructions (Millipore, Cat#: RMHMAG-84K)

Gastric emptying studies

Studies were conducted at RenaSci Ltd and adhered to the Animals Act 1986 and related UK legislation. 200-250g male rats (Charles River, UK) were maintained at 21±4^{o}C and 55±20% humidity on a normal 12 h light/dark cycle. Rats were randomized into weight-matched groups and fasted for ~23h prior to dosing. Water was withdrawn 1h prior to test compound administration. Rats were then administered bimatoprost by intravenous (i.v.) or oral routes (p.o.), as well as the control Loperamide 15 min prior to a test meal containing 0.05% w/v phenol red (Sigma-Aldrich; 1.5ml Po per rat) in 1.5% aqueous hydroxypropylmethyl-cellulose (HPMC) solution preheated to 37^{o}C. 15 min after dosing phenol red, animals were sacrificed by cervical dislocation. At sacrifice the abdominal cavity was opened, the pylorus and cardia of the stomach clamped to determine the amount of phenol red remaining in the stomach. The stomach was removed

and homogenized in 0.1N NaOH solution. Residual phenol red was quantified after filtration. The phenol red content from rats terminated immediately after administration were considered as the standard control i.e. maximal 100% absorbance. Accordingly, % emptying for each rat was calculated as: [1-(individual absorbance of the sample/mean absorbance of the standard control)] x 100.

Diet-induced obesity models

The cafeteria diet model was performed at Allergan Inc as described in (14). Briefly, Male Sprague-Dawley rats weighing ~200g and 6 weeks old were obtained from Harlan Laboratories (Dublin, VA, USA) and maintained on a standard chow for 2 weeks prior to randomization (by median group weights), group assignment and study. All studies were performed according to the requirements by Allergan Animal Use and Care Committee. Animals were divided by diet regimens of: standard chow-diet or cafeteria diet (CAF) in which the animals were free to select from three different energy dense/nutrient-poor high salt/low fiber human food items (obtained at Ralph's grocery chains) plus standard chow formula that would be varied daily. Caloric content and sources for both diet regimes are shown in Table S2; snack food components are detailed in Table S3. All foods were supplied ad libitum and water, with the snack food items varied daily according to fat, protein and carbohydrate contents as listed in Table S3.

Gross body weight was measured weekly through week 10. Within each diet regime, animals were assigned into drug and vehicle groups (n=8). 125 µL of drug or vehicle was topically administered daily over a 4 x 4 cm shaved area on the right side of the animal's abdominal segment (approximately 0.5 cm to the right of the spine and down the side).

Blood chemistry analysis

Blood was collected via submandibular or jugular vein of animals lightly sedated with isoflurane at 2-week intervals starting on week 0 through week 8 and divided into plasma and serum aliquots for subsequent analysis of glucose, insulin, lipoproteins (HDL and LDL/VLDL) and drug blood levels. Serum blood chemistry analysis was conducted using an ADCIA-1800 Chemistry Analyzer System (Siemens HealthCare Diagnostics, Tarrytown, NY, USA) using enzymatic and elimination/catalase assays (Glucose 103-358-91, Cholesterol 10376501, HDL 0-7511947, LDL B01-4760-01, TRIG B01-4133-01).

Histology

Animals were euthanized by CO_2 inhalation. Visceral fat weight was defined as the summed weight of collected intra-abdominal fat depots (i.e., gonadal, mesenteric, perirenal and retroperitoneal). Skin tissue samples were cut en bloc from treated and untreated sites fixed in PFA and processed for paraffin embedding. Tissue samples were recovered in a consistent mid-sagittal orientation perpendicular to the plane of the skin surface to provide reproducible tissue orientation. Adipose tissue area was determined by examining 4 slides from each section and measuring two 5mm areas per slide to obtain total area of adipose tissue per slide. Area measurements were combined for each rat and then group means were determined.

Statistical analysis

Statistical analysis was performed using Graphpad PRISM software as described in each figure and as appropriate for the data set generated. In most cases data were analyzed by 2-way ANOVA, and multiple comparison t-tests. In some figures, 1-way ANOVA was performed, with multiple comparison t-tests. In some figures, 2-way ANOVA and unpaired t tests were used. See figure legends for more details on each test performed on the dataset being presented.

Results

The metabolic effects of daily bimatoprost topical treatment over a 10-week period were investigated in rats fed a standard chow diet (Fig. 1). Compared to the vehicle group, bimatoprost reduced body weight gain in a dose dependent fashion yielding a 15% reduction at 10 weeks for the highest (3%) treatment group (Fig. 1A). Quantification of bimatoprost in the circulation and visceral fat at 10 weeks showed that the two efficacious doses of 1% and 3% that attenuated body weight gain translated into exposure ranges of 20-53.8 ng*h/ml and 980–5760 ng*h/g, respectively (Table S1). Otherwise, blood triglyceride concentrations were essentially unchanged throughout the trial (Fig. 1B). For the highest 3% bimatoprost treatment dose, analyses of circulating adipokine concentrations at 10 weeks showed no changes in leptin (Fig. 1C) but a reduction in total adiponectin compared to vehicle (Fig. 1D). All administered doses of bimatoprost decreased visceral fat content at 10 weeks compared to the vehicle group (Fig. 1E).

We next utilized indirect calorimetry to investigate how bimatoprost's effect of body weight was associated with energy expenditure and utilization. Daily topical treatment with 3% bimatoprost using separate cohorts of rats fed the standard chow diet again resulted in attenuated body weight gain (~ 7%) over a 9-week study period (Fig. 2A). Metabolic cage studies showed a reduction in whole animal 24hr energy expenditure at 9 weeks in the bimatoprost-treated group (Fig. 2B) consistent with the attenuated body weight gain. The respiratory exchange ratio (RER) was also decreased in the bimatoprost-treated group at 9 weeks indicating a metabolic shift towards utilization of fatty acid oxidation for energy (Fig. 2C) and consistent with reduced adiposity.

Next, we investigated whether bimatoprost was acting through metabolic effects involving either satiety or bioavailability. Consistent with our previous data, bimatoprost treatment attenuated weight gain in a separate animal cohort over a 10-week trial period (Fig. 3A) and this change was associated with a direct reduction in food intake (Fig. 3B). 24-hour indirect calorimetry, food intake, and fecal analyses were undertaken 1 week prior to bimatoprost or vehicle administration (week − 1), mid-way (week 4) and at the end of the study period (week 9). Here, cumulative food intake was not different at week 0 (Fig. 3C) but diverged in the bimatoprost-treated groups at week 4 (Fig. 3D) and week 9 (Fig. 3E). Analyses of total fecal output over each of these respective 24 h periods at weeks − 1, 4 and 9 showed reduced dry weight (Fig. 3F) and total energy content (Fig. 3G) in the bimatoprost-treated group. Fecal energy content normalized to body weight was unchanged (Fig. 3H) indicating that bioavailability was unaffected.

Consistent with the reduction in food intake and weight gain by bimatoprost, indirect calorimetry measurements performed over the same 24 h periods showed whole animal energy expenditure trended downwards at weeks 4 and 9 (Fig. S1A-C) together with a metabolic shift towards fatty acid utilization as evident after 4 weeks (Fig. S1D-F). Ambulatory movement was also not different between the two groups prior to bimatoprost-treatment at week − 1 or 4 with some divergence at 9 weeks (Fig. S1G-I). Overall, these findings show that bimatoprost treatment attenuates body weight gain in rats on the standard chow diet through a direct reduction in food intake.

Changes in food intake are often associated with altered hormone levels of ghrelin and GLP-1 (15, 16). Thus, we investigated whether gut hormones were affected following initial or subchronic daily bimatoprost exposure. Initial acute exposure (3 days) of rats to bimatoprost had no effect on circulating hormone concentrations (data not shown). However, in rats previously exposed to bimatoprost for a total of 7 days, there was a dramatic reduction in the circulating levels of several hormones. The highest impacts were on ghrelin, GLP-1, IL-6 with reductions of up to ~ 75%, 40%, and 50% respectively (Table I). PYY was also reduced, but only at the 60-min time point and in a non-dose-dependent manner.

Another mechanism controlling food intake is gastric motility. Satiety can be induced by a reduction of gastric motility inducing a sense of fullness (17). To investigate whether bimatoprost reduced gastric motility, rats were treated with bimatoprost either intravenously (iv.) or orally prior to a test meal to determine if drug treatment resulted in transient impairment (Fig. 4). This route of administration was chosen to examine the acute effects of drug treatment and the 1 mg/kg dose chosen was based on the exposure range that was achieved via topical administration (Table S1). Bimatoprost induced a significant reduction in gastric emptying at 1 mg/kg dosed iv. (~ 50%) or 10 mg/kg orally (~ 33%). The 1 mg/kg dose was as effective as the control μ-opioid agonist, loperamide (Fig. 4). Oral dosing was not as effective, likely due to the known poor oral bioavailability of bimatoprost.

We next examined whether bimatoprost attenuated body weight gain in rats maintained on an obesity promoting diet. For these studies we used a cafeteria (CAF) self-selection diet, allowing the animal to engage in hedonic feeding consistent with the hyperphagia that produces sustained neuronal alterations thought to underlie non-homeostatically regulated feeding behaviors associated with human obesity (18). This diet was chosen as it has been reported to result in rapid weight vs. a traditional high-fat diet, although due to the complexities of the diet we were unable to perform food intake or energy expenditure analyzes. Rats were started on their respective diets, a standard chow or cafeteria diet (CAF), concurrently with topical administration of different doses of bimatoprost (Fig. 5).

The CAF diet induced a body weight gain of 85% (~ 295 g) compared to 65% (~ 220 g) for the typical standard chow diet over the 10-week trial (Fig. 5A versus Fig. 1A). Daily topical treatment with bimatoprost again attenuated body weight gain in a dose dependent manner with a maximum 23% reduction in body weight gain at the 3% dose (Fig. 5A). The lowest efficacious dose was 1% bimatoprost, which translated into approximately 29.7 ng*h/ml exposure in blood and 2850 ng*h/g in visceral fat after 10 weeks (Table S1). These findings suggest that bimatoprost's effect on body weight gain was not influenced by the diet.

Glucose tolerance testing was not performed as a diabetic state in this rat strain reportedly occurs between 15–26 weeks on this diet (14).

While the cafeteria diet evoked a sustained and progressive elevation of circulating triglyceride concentrations (454 mg/dl), these effects were dose-dependently blunted by bimatoprost treatment (max effect, 152.5 mg/dl) (Fig. 5B) with no changes in circulating LDL and HDL (data not shown). Also associated with elevated serum triglycerides due to the CAF diet in the vehicle group was an increase in total serum leptin (Fig. 5C vs. Figure 1C) and adiponectin (Fig. 5D vs. Figure 1D). The increase in circulating leptin concentrations in CAF-diet fed rats was largely unchanged by bimatoprost treatment (Fig. 5C). However, 3% bimatoprost treatment dramatically attenuated the CAF diet-induced adiponectin increase (Fig. 5D). Consistent with the effects of obesity-promoting diets, the CAF dietary regime increased visceral fat content across all groups compared to rats fed the standard chow diet (Fig. 5E vs. Figure 1E). Nevertheless, 3% bimatoprost decreased this content at 10 weeks compared to the vehicle group (Fig. 5E). Overall, the increased concentrations of leptin and total adiponectin in response to the CAF-diet is consistent with white adipose tissue expansion that occurs over a period of 6–20 weeks in rat models of dietary-induced obesity (19, 20). Bimatoprost also attenuated the obesity-promoting effects of the CAF-diet.

Our earlier experiments established that the bimatoprost-induced changes in body weight gain correlated with systemic drug levels. To explore whether there were also local effects on fat content, the effects of bimatoprost on local subcutaneous (SQ) fat directly below the dosing area was investigated in CAF-fed rats (Fig. 6). Upon sectioning, it was apparent that both intra-dermal fat (within the dermis) and SQ fat depots existed. Because these two fat depots are likely differentially regulated (21) they were analyzed separately (representative pictures in Figure S2). Quantitative analyses indicated that both intradermal and SQ fat content were reduced in rats treated with 3% bimatoprost as compared to vehicle-treated rats irrespective of the dosed or non-dosed area on the treated animals (Fig. 6). This finding suggests that SQ adiposity was reduced uniformly and driven by systemic action of bimatoprost.

Discussion

Despite its reported anti-adipogenic actions, we found that bimatoprost evoked beneficial metabolic effects *in vivo* via an inhibition on food intake. This resulting decrease in energy consumption translated into a decrease in body weight gain. In addition to a decrease in body weight gain, metabolic cage studies demonstrated that treatment with bimaotoprost was associated with a metabolic switch to fatty acid oxidization.

Investigation of the food intake reduction led to the discovery of a bimatoprost-induced profile of reduced circulating GLP-1, ghrelin, and PYY, which modulate gastric motility and food intake (22, 23). By comparison, the reduction in circulating PYY and GLP-1 would be expected to promote food intake, however, these changes are likely secondary to the more profound reduction in ghrelin levels, which could account for the increase in satiety (Table I. In support of these changes, gastric emptying was decreased by bimatoprost treatment (Fig. 4). While it is not proven, the delayed onset of the hormone changes,

compared to the relatively quick onset of gastric mobility reduction, suggests that the effects on mobility precede the broader changes in gut hormone levels. Gastric restriction as clinically manifested in bariatric surgery or banding has been shown to not only lead to weight loss, but also alters levels of circulating satiety hormones (24).

These newly described effects of bimatoprost on gastric motility and satiety suggests a novel biological role for its biological counterpart, prostamide F2α. It is currently established that prostamide F2α and its precursor, anandamide, exert opposing effects on adipogenesis. These actions are exemplified in mice administered obesity promoting diets where increased local anandamide production within adipose stores promotes adipogenesis while prostamide F2α levels are lowered (4). Our findings suggest this reciprocal relationship extends to effects on gastric emptying, ghrelin secretion, and food intake, which are increased by anandamide through actions on the brain (7). Thus, bimatoprost may be pharmacologically mimicking an endogenous action of prostamide F2α that opposes anandamide action in the gastrointestinal tract and adipose tissue to balance caloric intake with energy storage.

The major receptor for bimatoprost and prostamide F2αis the prostamide receptor, which is a variant form of the FP receptor (25). It has been pharmacologically identified by specific receptor antagonists that block its actions in primates in vivo, but not those of FP agonists. It is unclear if the systemic effects of bimatoprost are the result of prostamide receptor signaling, but recently bimatoprost has been described to act as a TRPA1 agonist on trigeminal ganglion neurons in vitro, potentially identifying a new systemic target (26). Interesting the TRPA1 agonist, cinnamaldehyde, shows a similar impact on food intake and gastric emptying as bimatoprost (27).

In summary, our findings show that systemic bimatoprost treatment regulates energy homeostasis. Prostamide F2α inhibits adipogenesis through a feed-forward mechanism to maintain adipose homeostasis under steady-state conditions (4). The present findings suggest there may be a broader orchestrated role for prostamide F2α antagonism of the endocannabinoid mechanistic axis regulating satiety as well. It is possible that exploiting prostamide action could provide an alternative pathway toward therapeutic intervention of obesity and related metabolic complications.

Abbreviations

IL – InterleukinCB1 – Cannabinoid receptor 1

GLP-1- Glucagon-like peptide-1

HDL – High density lipoprotein

LDL – Low density lipoprotein

PTGS – prostaglandin-endoperoxide synthase

PYY - peptide tyrosine tyrosine

TRPA1 – transient receptor potential ankyrin 1

VLDL – Very low-density lipoprotein

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

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Competing Interests:

Although no financial gain or loss is expected due to this publication, we feel it is important to declare the following: Neil Poloso, Iona Raymond, Warren Tong are employees of Allergan. Neil Poloso, Warren Tong hold stock and/or stock options in Allergan. Kerry Loomes received research funding from Allergan for work described in this manuscript.

Contributions: CS, CV, IR, CC, CW, NJP conducted the experiments and acquired the data. CS, NJP, DFW, IR, CW, KL designed the research studies. NJP, KL, CS, IR, CW, WT analyzed the data. NJP, KL, DFW wrote the manuscript.

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Tables

Table 1. hormone-induced changes following sub-chronic treatment with bimatoprost in standard chow-fed rats.

	30 min.				60 min.			
Hormone (pg/ml)	Control (N=8)	Bimatoprost (N=8/8/8)			Control	Bimatoprost (N=8/8/8)		
		3%	10%	25%		3%	10%	25%
Ghrelin	44±30	19±18	17±16*	18±12*	77±66	51±51	43±33	23±25*
GIP	19±7	19±7	21±11	20±7	15±6	14±6	16±8	18±7
Leptin	533±192	736±205	572±175	494±276	360±99	543±261	427±148	522±343
GLP-1	133±12	124±14	120±29	112±15$^{\#}$	130±20	91±24**	79±28***	113±5
PYY	88±21	82±5	73±19	73±12	77±34	56±16	37±23**	50±19
Insulin	345±154	467±196	423±328	475±250	378±185	450±306	309±312	460±276
Glucagon	30±2	30±3	28±4	27±2	24±4	19±4	17±4	20±5
Amylin	53±5	53±4	52±4	48±5	52±8	45±8	44±5	48±8
IL-6	710±117	586±117	591±210	518±124	730±255	391±226**	344±145**	492±139

Separate cohorts of rats received daily administration of either vehicle or one of three bimatoprost doses for four days. Rats were fasted overnight for 16 hours and administered a final dose of either vehicle or bimatoprost. Blood samples were collected at 30 min and 60 min post-administration. Data denotes mean ± SD. Statistical significance was determined by 1-way ANOVA and post-hoc t-tests. *P<0.05, **P<0.01, ***P<0.001, $^{\#}$P<0.05 (unpaired t-test) *versus* the respective 30 min or 60 min control. Individual samples with measured hormone levels below detectable limits are reported as standard curve-derived minimum threshold values.

Figures

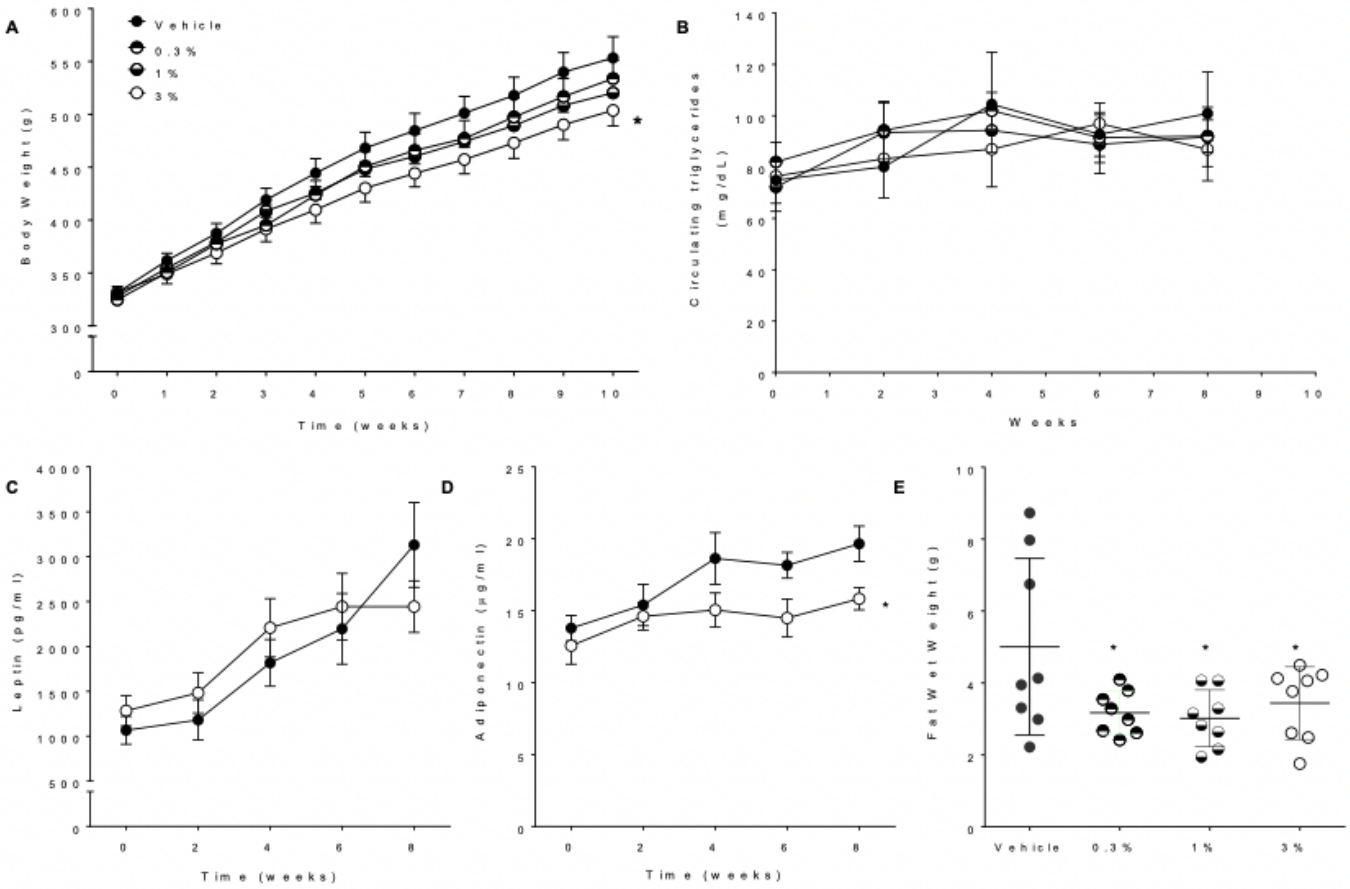

Figure 1

Bimatoprost inhibits weight gain and in rats fed a standard chow diet in a dose dependent manner. (A). Body weight trajectories following topical application of bimatoprost (BIM) to a 4x4 cm2 area on the dorso-lateral side. Body weight was measured weekly at the same time of day and values are mean ±SEM (n=8). Plasma and serum were isolated from rats at week 8 and analyzed for circulating triglycerides (B), leptin [diluted 1:500] (C) and adiponectin [1:5] (D) using Millipore kits (RADPK-81K-ADPN and RADPK-81K) with each sample run in duplicate. Data for each group of animals for each time point were generated, averaged and plotted as mean +/- SEM per time point. One of 2 similar experiments shown. (E). Wet visceral fat content was excised and weighed. Statistical analyses were performed by either one-way ANOVA with Boneforri's post-hoc test or two-way ANOVA performed with multiple comparisons correcting with Dunnett's post-test. *p<0.05, **p<0.01.

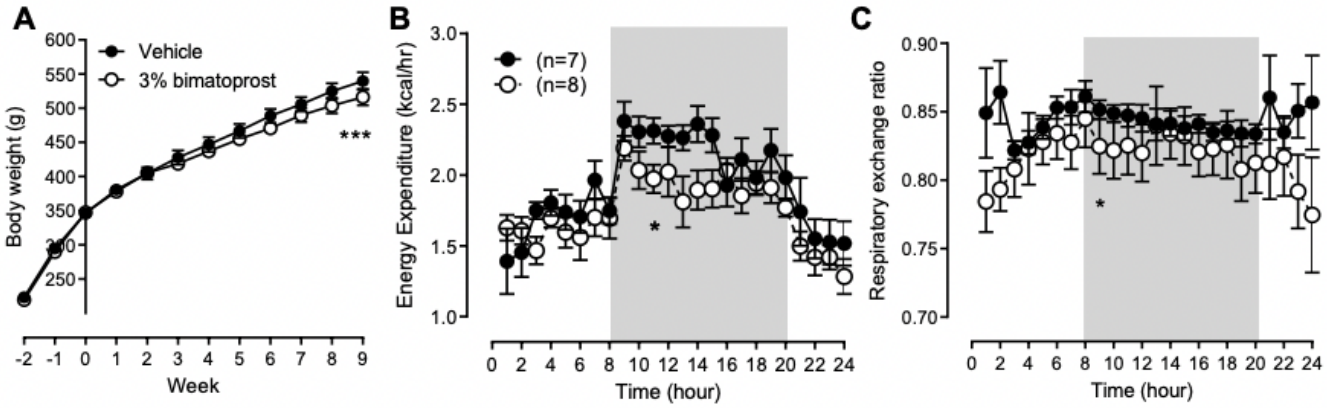

Figure 2

Bimatoprost attenuates body weight gain in rats in association with decreased energy expenditure and a metabolic shift toward fat oxidation. Rats were administered either vehicle (n=12) or 3% bimatoprost (n=12) for 9 weeks. Body weights for vehicle and treated groups at week 0 were 223 ± 13g and 220 ± 8g respectively (mean ± SD, P = NS). For indirect calorimetry measurements at 9 weeks individual rats were acclimated to respirometry chambers for approximately 72 h followed by a 24-h period of data collection. (A) Body weight trajectories (time* bimatoprost treatment interaction: *** P<0.0001). (B). 24 hr energy expenditure measurements (bimatoprost treatment effect: * P<0.05). (C). Respiratory exchange ratio (time*bimatoprost treatment interaction: * P<0.05). Statistical significance was analyzed by repeated measures two-way ANOVA with Sidak's post-hoc multiple comparisons test. All values expressed as mean ± SEM.

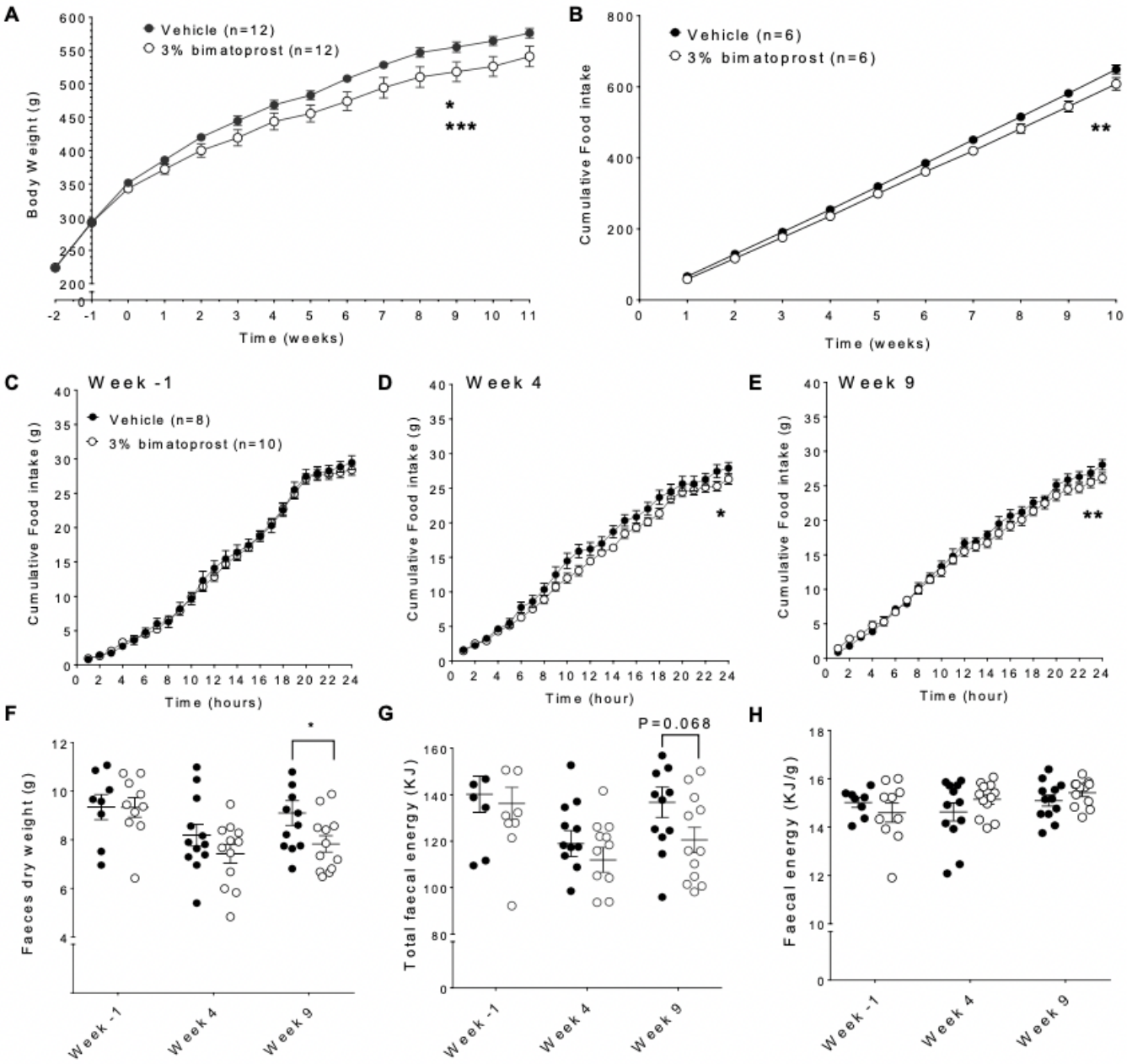

Figure 3

Bimatoprost promotes satiety in rats fed a standard chow diet. Rats were administered either control (●) or 3% bimatoprost (●) for 11 weeks. Animal weights for control and treated groups at week 0 were 343 ± 20g and 352 ± 18g respectively (mean ± SD, P = NS). (A) Body weight trajectories (time*bimatoprost treatment interaction: $p<0.0001$; bimatoprost treatment effect: $p<0.05$). (B) Cumulative food intake measured per cage (2 rats/cage; time*bimatoprost treatment interaction: $p<0.01$). (C-E). Cumulative food intake over 24h at weeks -1, 4 and 9 respectively. Weeks 4 and 9 show a time*bimatoprost treatment interaction ($p<0.05$ and $p<0.01$ respectively). (F-H). Analyses of faeces collected over the respective 24-hour periods at weeks -1, 4 and 9. Statistical significance for A-E was analyzed by repeated measures two-

way ANOVA and F-H by unpaired t-tests. All values expressed as mean ± SEM. * P<0.05; ** P<0.01; ***P<0.0001

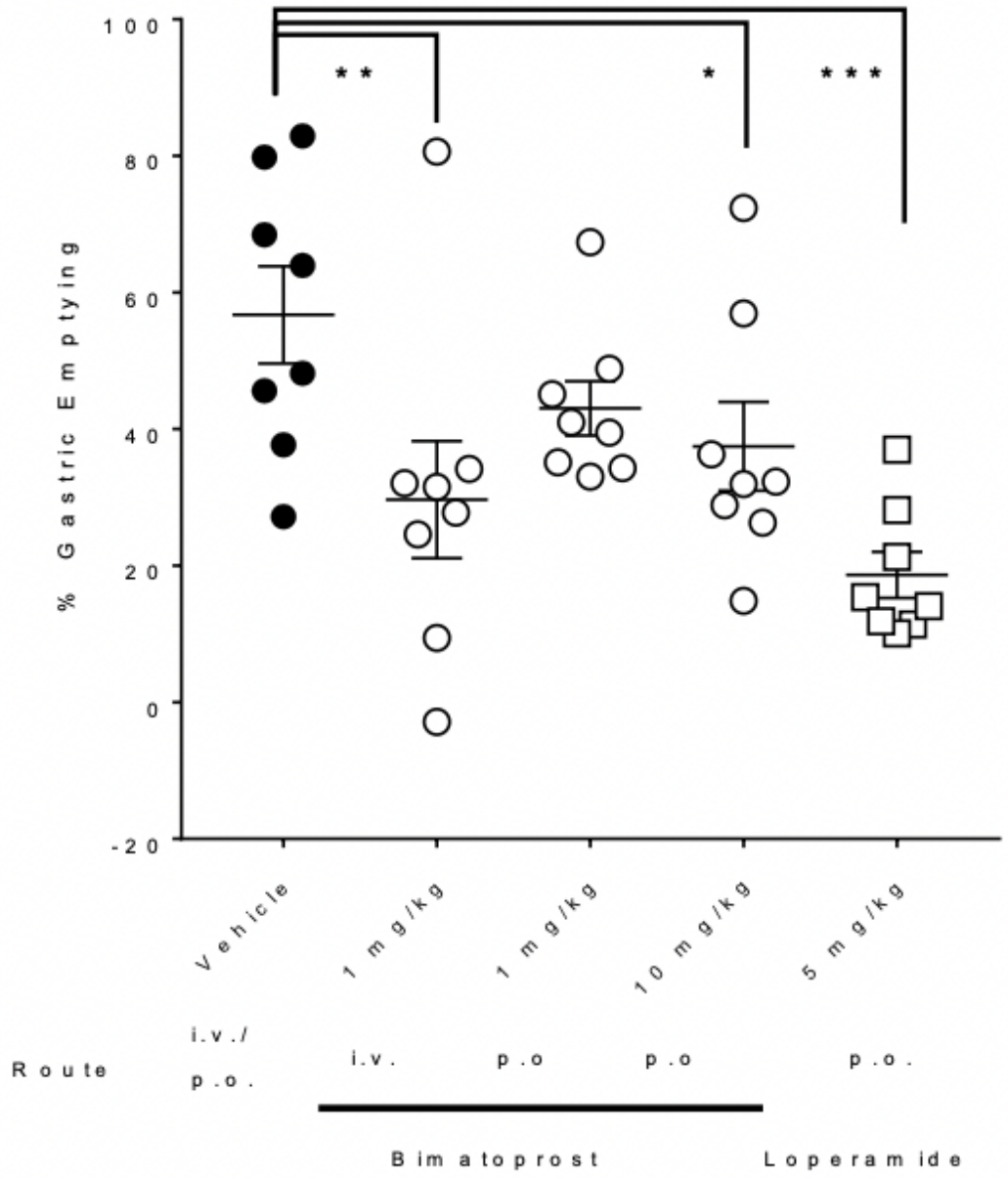

Figure 4

Gastric emptying is impacted by acute bimatoprost treatment. The impact of bimatoprost on gastric emptying in rats (phenol red administration assay) was measured after administration by intravenous (i.v.) or oral (p.o.) administration. Loperamide was compared as a positive control. Percentage emptying for each rat was calculated as: [1-(individual absorbance of the sample/mean absorbance of the standard control)] x 100. data is shown as mean ± SEM for each group (n=8). Statistical significance was determined by 1-way ANOVA, *p<0.05, ** p<0.01, ***p<0.001.

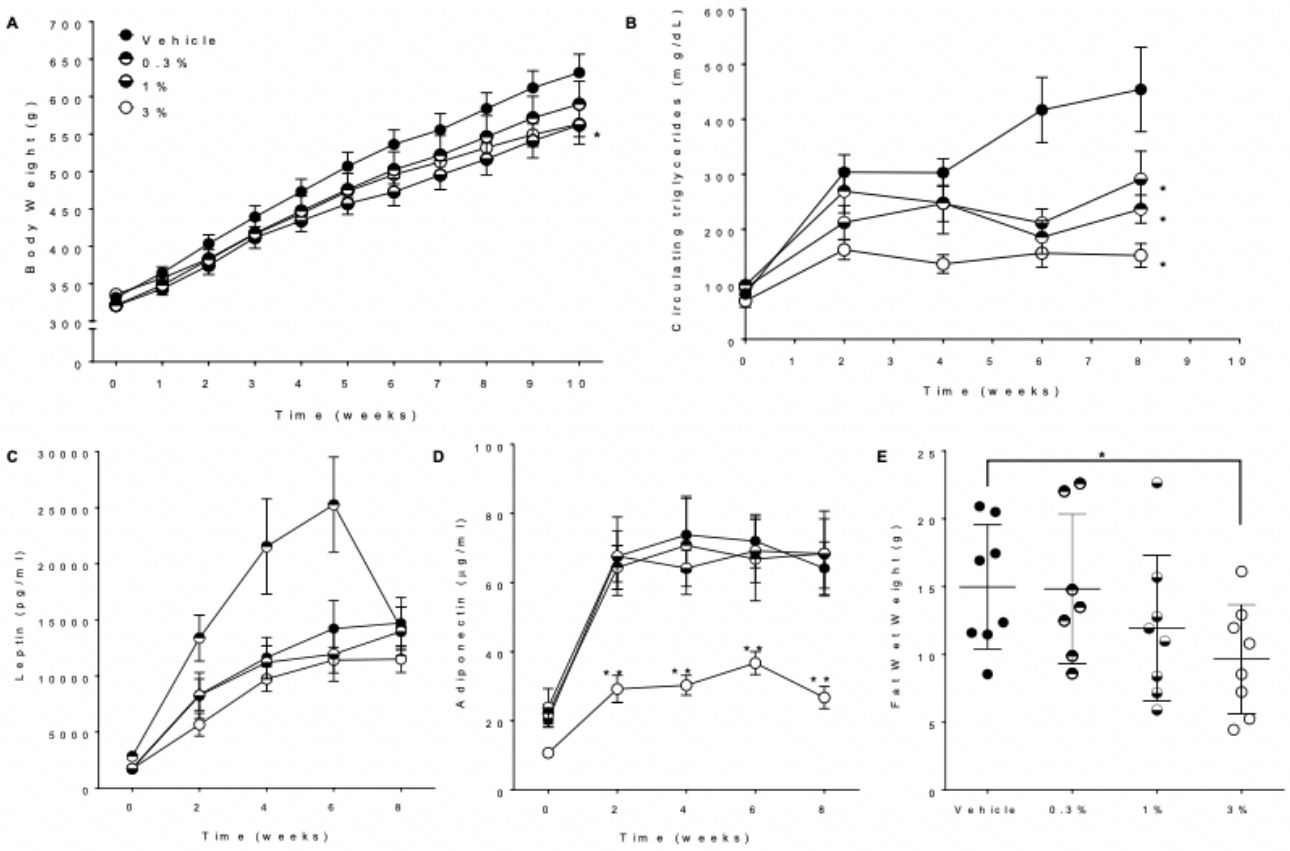

Figure 5

Bimatoprost inhibits weight gain and measures of obesity in rats fed a cafeteria diet in a dose dependent manner. (A). Body weight trajectories following topical application of bimatoprost (BIM) to a 4x4 cm2 area on the dorso-lateral side. Body weight was measured weekly at the same time of day and values are mean ±SEM (n=8). Plasma and serum were isolated from rats at week 8 and analyzed for circulating triglycerides (B), leptin [diluted 1:500] (C) and adiponectin [1:5] (D) using Millipore kits (RADPK-81K-ADPN and RADPK-81K) with each sample run in duplicate. Data for each group of animals for each time point were generated, averaged and plotted as mean +/- SEM per time point. One of 2 similar experiments shown. (E). Wet visceral fat content was excised and weighed. Statistical analyses were performed by either one-way ANOVA with Boneforri's post-hoc test or two-way ANOVA performed with multiple comparisons correcting with Dunnett's post-test. *p<0.05, **p<0.01.

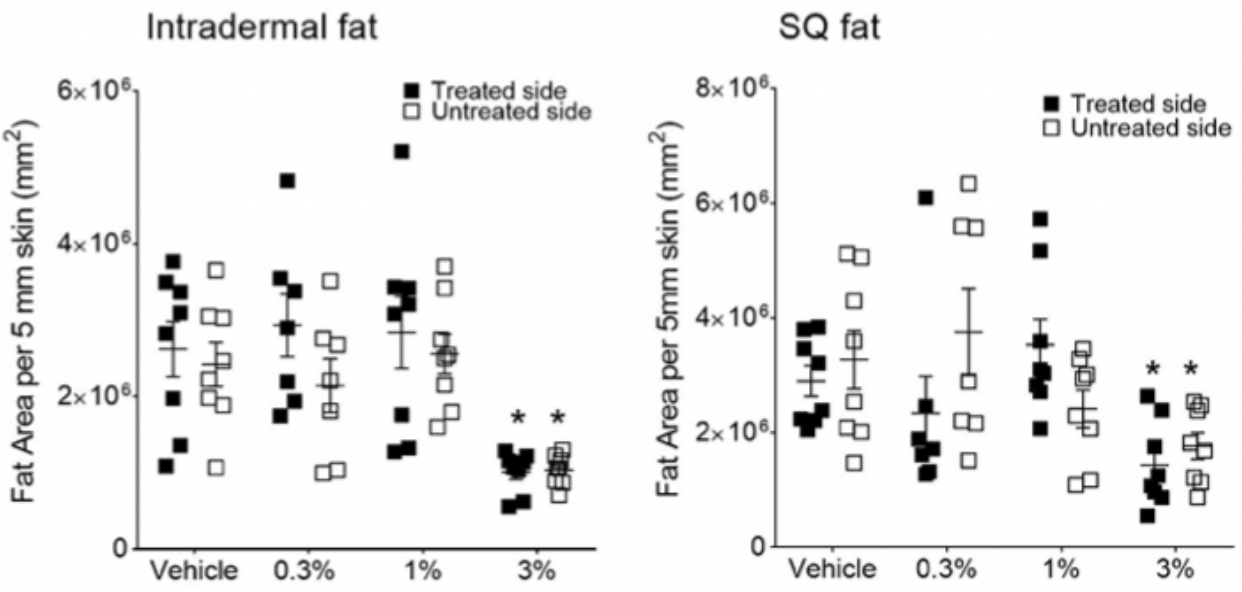

Figure 6

Bimatoprost reduces local intradermal and subcutaneous fat in CAF-diet fed rats. Tissues under the treatment area as well as under an untreated area (opposite side) were recovered at 10-weeks after CAF-diet treatment, fixed, paraffin-embedded and sectioned. Area of cumulative adipose cells was measured per 5mm of skin in two locations beneath the skin: intra-dermal and SQ fat by quantitative image analysis. Mean total adipose tissue per group is shown ± SEM. Statistical significance calculated by 2-tailed T-tests vs. vehicle treated. N=8 rats per group (0.3% group, n=7).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigureMMApr2020.docx
- MMSupplementalmethods.docx