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# Knockdown of central ghrelin O-acyltransferase by vivo-morpholino reduces body mass of rats fed a high-fat diet

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ABSTRACT

The enzyme ghrelin O-acyltransferase (GOAT) activates the orexigenic peptide ghrelin by transferring an acyl group from fatty acids to the serine-3 residue of the ghrelin molecule. This allows ghrelin to bind to its only known receptor, the growth hormone secretagogue receptor type 1a (GHSR1a). While studies have examined the hypothalamic transcriptional response of GOAT to metabolic challenge in mice, little has been examined in the rat hypothalamus. Furthermore, it has not been possible to identify the role of central GOAT separate from that of the periphery, since previous studies either knocked out GOAT system-wide or administered a GOAT inhibitor intraperitoneally. To determine if central GOAT expression is modulated by changes in energy state, we subjected rats to either forty-eight hours of food deprivation or three weeks of food restriction and found that GOAT mRNA increases significantly in both the hypothalamus and the stomach fundus in response to both metabolic challenges. We also found increases in hypothalamic ghrelin mRNA and stomach GHSR1a mRNA in response to food deprivation, as well as increases in hypothalamic GHSR1a mRNA in response to food restriction. We then conducted a second study where we continuously infused amorpholino antisense oligonucleotide into the lateral ventricles of rats to knock-down GOAT centrally while the animals were exposed to a high fat diet. Our results show that rats receiving the GOAT antisense gained less weight, and decreased their caloric efficiency when eating a high fat diet compared to control animals. These data suggest that central GOAT plays a role in modulating metabolism in rats.



## Introduction

Ghrelin is a metabolic hormone produced primarily by the stomach. The metabolic effects of ghrelin include stimulation of growth hormone secretion [15,21,31], increases in food intake and adiposity [28,31], as well as modulation of glucose [11] and lipid metabolism [27]. Much of ghrelin's biological activity depends on an esterification reaction in which a fatty acyl side chain is linked to the serine-3 residue, resulting in an acylated ghrelin molecule [15]. This side chain, typically but not exclusively involving an 8-carbon octanoyl moiety, is primarily supplied through dietary lipids [13,20]. Without this activation, ghrelin cannot bind to its receptor, the growth hormone secretagogue receptor type 1a (GHSR1a) [12,32]. The esterification reaction is accomplished through the activity of the enzyme ghrelin O-acyltransferase (GOAT), a member of the group of membrane-bound O-acyltransferases [12,32]. Transcripts for GOAT have been found in the stomach [8,9,24,25,32] and hypothalamus of both rats and mice with widespread expression in humans [16]. Furthermore, ghrelin is the only known hormone requiring octanoylation to be activated [15], making GOAT a desirable target to specifically reduce acylated ghrelin levels and affect such phenomena as food intake and adiposity.

The effects of ghrelin on food intake and adiposity have been attributed to the action of peripheral ghrelin on its receptors in the brain, and particularly in the hypothalamus [28,30,31]. There is some debate on the origin of ghrelin binding to hypothalamic and extrahypothalamic target sites, with some authors suggesting that ghrelin is produced within the hypothalamus itself [3,15,17]. While the existence of a hypothalamic source of ghrelin has been questioned [7], one would expect that if ghrelin was secreted in the hypothalamus, GOAT would also be synthesized in hypothalamic tissues, and that the expression of this enzyme would be modulated by changes in energy state.

The response of the ghrelin system to short-term food deprivation in mice (in both the stomach and the hypothalamus) as well as to chronic food restriction in rats (in the stomach only) has

*Abbreviations:* GOAT, ghrelin O-acyltransferase; GHSR1a, growth-hormone secretagogue receptor type 1a; HFD, high-fat diet.

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been reported previously [8–10,13,18]. Particularly interesting is the fact that GOAT mRNA expression is increased in the hypothalamus of fasted rats [8] although it is not clear if similar increases in the expression of GOAT in prolonged energy challenges such as chronic food restriction. Nevertheless, the fact that hypothalamic GOAT mRNA expression can be modulated by fasting suggests that this enzyme is important for the regulation of energy balance. Indeed, the expression of GOAT has been linked to changes in energy state. For instance studies using mice with targeted deletion of the GOAT gene have shown that these mice, like GHSR and ghrelin KO mice, show mild alterations in body weight and food intake [17]. These differences however, are more evident when GOAT KO mice are exposed to high fat diets or when exposed to fasting conditions [13,33]. Interestingly, a small-molecule GOAT-inhibiting drug named GO-CoA-Tat, reduced the excessive weight gain observed in wild-type C57BL6 mice on a diet high in medium-chain triglycerides [1]. This reduction in weight occurred quite rapidly, taking only one week for significant differences to occur, and divergence appearing to begin only after about four days on the diet. Nevertheless, while GO-CoA-TAT treated mice are leaner than vehicle treated animals, it is not possible to determine the relative contribution of GOAT produced in the hypothalamus versus that of GOAT synthesized in the stomach in these processes.

Given that the effects of total genetic deletion of GOAT, and those of GO-CoA-Tat are observable in animals exposed to diet high in triglycerides, here we attempted to further characterize the role of central GOAT on body weight gain and caloric intake in rats exposed to a high fat diet. In the current study, we sought out to first confirm regulation of ghrelin related genes by measurements of ghrelin, GHSR1a and GOAT mRNA in the rat fundus and hypothalamus in response to forty-eight hours of food deprivation and three weeks of food restriction. After verifying that GOAT mRNA is present and can be regulated in the hypothalamus, we then sought out to determine the effects of knocking down GOAT mRNA expression in the brain on measures of food intake and body weight in rats. We accomplished this by providing continuous infusion of vivo morpholino antisense oligonucleotides (VMO) targeting the rat GOAT sequence for a four-week period into the left lateral ventricle of rats.

## Materials and methods

### *Experiment 1: Effect of food deprivation and food restriction on GOAT, ghrelin, and GHSR1a mRNA in the fundus and the hypothalamus*

#### *Animals*

Male Long Evans rats ($N = 50$) (276–300 g) were obtained from Charles River (St.-Constant, Quebec), and kept individually in a 12/12 light/dark cycle at 21 °C, with constant access to standard chow and water before experimental manipulations. After a one-week baseline where food and body weight were recorded, rats were randomly assigned to one of three groups: (1) Control ad lib. fed rats ($n = 17$); (2) rats food deprived for 48 h ($n = 16$); and (3) rats food restricted for three weeks ($n = 16$). Food restricted animals were kept on a diet consisting of 50% of the average daily food consumed during baseline. All experimental manipulations were approved by the Carleton University Animal Care Committee and adhered to the standards of the Canadian Council for Animal Care.

#### *Tissue collection*

Animals in all groups were rapidly decapitated and hypothalamic punches (relative to bregma: AP −2.3 mm to −3.8 mm, L −1.0 mm to +1.0 mm, DV +7.5 mm to base) were extracted from the brain and placed in TriZol (Invitrogen). Stomach fundus samples were also obtained. Brain samples were stored in TriZol at −20 °C and fundus samples at −80 °C (not in TriZol) until processed. Trunk blood was collected in BD Vacutainer K2EDTA tubes (Franklin Lakes, New Jersey). Blood samples were briefly vortexed, centrifuged at 3000 RPM for 15 min in an IEC Centra GP8R centrifuge. Blood plasma was separated and treated with 5 μl 1 N HCl and 5 μl p-hydroxymercuribenzoic acid per 0.5 ml of plasma to protect against degradation of acylated ghrelin.

#### *Enzyme-linked immunosorbent assay*

Enzyme-linked immunosorbent assay was conducted on all plasma samples using a Millipore (Billerica, MA) Rat/Mouse Ghrelin (Active) ELISA kit according to the manufacturer's instructions. All samples were run in duplicate in a single assay. Intra assay variability was less than 10%.

#### *RNA extraction and cDNA synthesis*

RNA extraction was performed on all brain and stomach samples obtained in both experiments using the TriZol protocol provided by Invitrogen, using 13 μl of linear acrylamide as a co-precipitate with isopropyl alcohol during the RNA precipitation stage. cDNA synthesis was performed on all mRNA samples. Briefly, 1 μl oligo(dT) primer (Invitrogen) was added to 9 μl mRNA and heated at 70 °C for 5 min. To each sample, a master mix of 4 μl 5× first strand buffer (Invitrogen), 2 μl 0.1 M DTT (Invitrogen), 1 μl 10 mM dNTP (Invitrogen), 1 μl RNase inhibitor (Promega), 1 μl SS2 RT (Invitrogen), and 1 μl DEPC-treated water were added. Samples were then run on a PTC-200 Thermal Cycler (MJ Research) at 42 °C for 1.5 h followed by 90 °C for 10 min. Samples were maintained in the cycler at 4 °C until stored at −20 °C.

#### *Quantitative RT-PCR*

Quantitative RT-PCR was conducted on all cDNA samples in duplicate to determine fold changes in GOAT, GHSR1a, and ghrelin mRNA levels, using the $2^{-ddCt}$ method with the ad lib. group as a baseline control group. Briefly, 5 μl of each cDNA sample was added to separate wells in a PCR plate. 2 μl of 2 μM Primer, 3 μl of milli-Q water, and 10 μl iQ SYBR Green Super Mix with fluorescein (Bio-Rad) were added to each well. The plate was run on a MyiQ Single Color Real-Time PCR Detection System (Bio-Rad) for 30 s at 95 °C, followed by 45 cycles of the following settings: 10 s at 95 °C for denaturing, 30 s at 55 °C for annealing, and 20 s at 72 °C for extension. The plate was then run for 1 min at 95 °C and 1 min at 55 °C. A melt curve was constructed by running the plate for 30 s at 55 °C for 41 repeats, increasing the temperature by 1 °C for each cycle. β-Actin and GAPDH were used as housekeeping genes. Primers used were as shown in Table 1.

### *Experiment 2: Effect of central GOAT knock-down on body weight and food intake*

#### *Animals*

Male Long Evans rats ($N = 32$) (276–300 g) were obtained from Charles River (St.-Constant, Quebec), and kept in a 12/12 light/dark cycle at 21 °C. All rats were housed individually and given ad lib. access to a high fat diet consisting of 60% kcal from fat (TD.06414; Harlan Laboratories, Indianapolis, IN) with constant access to water throughout the experiment. Following a one-week baseline, animals were weight-matched and assigned to one of four groups: (1) vehicle control ($n = 7$), (2) reverse control ($n = 6$), (3) negative control ($n = 7$), and (4) GOAT anti-sense treatment ($n = 6$). Six animals were removed from the experiment due to experimental attrition (four due to surgical complications and two due to misplaced cannulae). All experimental manipulations were approved by the Carleton University Animal Care Committee and adhered to the standards of the Canadian Council for Animal Care.

**Table 1**
Primers used for qRT-PCR.

| Gene | Primer sequence |
|---|---|
| Ghrelin | |
| Forward | 5′-CCAGAGGACAGAGGACAAGC-3′ |
| Reverse | 5′-AGTTGCAGAGGAGGCAGAAGCT-3′ |
| GOAT | |
| Forward | 5′-TGCCACCTGGGTCTTCACTAC-3′ |
| Reverse | 5′-ACGCTGCCTCCACCTTCC-3′ |
| GHSR1a | |
| Forward | 5′-CCAGAACCACAAGCAGACAGTG-3′ |
| Reverse | 5′-GAAGAGGACAAAGGACACCAGG-3′ |
| NPY | |
| Forward | 5′-TGGACTGACCCTCGCTCTAT-3′ |
| Reverse | 5′-GTGTCTCAGGGCTGGATCTC-3′ |
| β-Actin | |
| Forward | 5′-TCATGAAGTGTGACGTTGACATCCG-3′ |
| Reverse | 5′-CCTAGAAGCATTTGCGGTGCACGATG-3′ |
| GAPDH | |
| Forward | 5′-AAGATGGTGAAGGTCGGTGT-3′ |
| Reverse | 5′-CTTGCCGTGGTAGAGTCAT-3′ |

*Vivo-morpholinos*

Vivo-morpholinos were designed by and obtained from Gene Tools LLC (Philomath, OR). Treatment vivo-morpholinos were designed based on accession number NM_001107317.2 (*Rattus norvegicus* membrane bound O-acyltransferase domain containing 4 (Mboat4), mRNA), with the sequence 5′-GAAAGAAGAACTGGAGCCAATCCAT-3′. This VMO was designed to target the 5′ end, including the start codon, of the GOAT transcript. The reverse of this sequence was used as a reverse control. In addition, a negative control targeting a human beta-globin intron mutation with the sequence 5′-CCTCTTACCTCAGTTACAATTTATA-3′ was used. Vehicle animals were given sterile PBS at a pH of 7.35. All vivo-morpholinos were diluted to a concentration of 35.90 μM in sterile PBS and stored at room temperature until used. A pilot study determined that this VMO was effective in reducing hypothalamic GOAT mRNA expression to an average of 40.69% ± 14.6%, and neuropeptide Y (NPY) mRNA expression to an average of 63.42% ± 8.86% of vehicle infused animals. Similarly, the VMO reduced GOAT and NPY expression to 45.15% ± 16.20 and 28.48% ± 10.22 of the reverse sequence morpholino (negative control). No differences in hypothalamic expression of GOAT or NPY mRNA between rats treated with vehicle and reverse sequence morpholino negative controls.

*Surgery*

Following the one-week baseline, surgery was performed for continuous infusion of vehicle or the corresponding vivo-morpholino, at 0.25 μl/h, by way of subcutaneous 4-week Alzet osmotic mini-pumps obtained from DURECT Corporation (Model 2004; Cupertino, CA). Animals were anesthetized with isoflurane supplemented with oxygen and given 0.1 ml subcutaneous Metacam prior to surgery. Mini-pumps were attached via tubing to a cannula targeting the left lateral ventricle (relative to bregma: AP −0.08 mm, L +0.16 mm, DV −0.36 mm). Animals were given a two-day recovery period before being handled again. Placement into the ventricles was verified by injection of a blue dye and confirmed by the presence of the dye in the third ventricle.

*Mass and food intake measurements*

Day of surgery was identified as day 0. Food intake and rat mass were measured daily at noon from day 0 up to day 17. Total weight gain and total cumulative food intake were calculated relative to the end of the two-day surgical recovery period (day 2). Repeated measures two-way ANOVAs followed by one-way between groups ANOVAs and Fisher's LSD post hoc tests were conducted separately for each day to compare, between groups, total percentage weight gain as well as total food intake relative to rat mass at day 2, as well as total weight gain over total food intake.

## Results

*Experiment 1: Effect of food deprivation and food restriction on GOAT, ghrelin, and GHSR1a mRNA in the fundus and the hypothalamus*

*Effect of food restriction and deprivation on hypothalamic GOAT, ghrelin, and GHSR1a mRNA*

One-way ANOVAs indicated that differences occurred in the hypothalamus for GOAT mRNA ($F_{2,45} = 5.466$, $p < 0.05$), ghrelin mRNA ($F_{2,46} = 4.573$, $p < 0.05$), and GHSR1a mRNA ($F_{2,38} = 13.648$, $p < 0.05$) between the ad lib., 48 h food deprived, and three week food restricted groups (see Fig. 1A–C). Fisher's post hoc tests revealed that in the hypothalamus, GOAT mRNA was significantly elevated after 48 h of food deprivation and after 3 weeks of food restriction ($p < 0.05$) compared to ad lib. animals. While hypothalamic ghrelin mRNA levels were significantly elevated after 48 h of food deprivation ($p < 0.05$), hypothalamic ghrelin mRNA expression was not elevated after 3 weeks of food restriction ($p > 0.05$) in relation to control ad lib. fed rats. GHSR1a mRNA levels in the hypothalamus did not change in response to 48 h of food deprivation but increased in response to three weeks of food restriction ($p < 0.05$).

*Effect of food restriction and deprivation on stomach fundus GOAT, ghrelin, and GHSR1a mRNA*

One-way ANOVAs indicated that differences occurred in the stomach fundus for GOAT mRNA ($F_{2,38} = 4.949$, $p < 0.05$) and for GHSR1a mRNA ($F_{2,36} = 7.350$, $p < 0.05$), but not for ghrelin mRNA between the ad lib., 48 h food deprived, and three week food restricted groups (see Fig. 1D–F).

In the stomach fundus, GOAT mRNA levels followed a similar pattern as in the hypothalamus. Fisher's LSD post hoc tests revealed that GOAT mRNA significantly increased after 48 h of food deprivation ($p < 0.05$) as well as after 3 weeks of food restriction ($p < 0.05$). GHSR1a mRNA levels in the fundus increased relative to ad lib. animals in response to 48 h of food deprivation ($p < 0.05$) but no change was found relative to ad lib. animals in the three week food deprived animals.

*Effect of food restriction and deprivation on circulating acylated ghrelin levels*

A one-way ANOVA ($F_{2,38} = 15.413$, $p < 0.001$) followed by Fisher's LSD post-hocs revealed that plasma levels of active ghrelin were increased in 3-week food restricted animals 9.6 fold relative to ad lib. animals (Fig. 2). No significant difference was found between 48-h food-deprived animals and ad lib. animals.

*Experiment 2: Effect of central GOAT knock-down on body weight and food intake*

*Effect of drug treatment on hypothalamic GOAT and NPY mRNA expression*

*Effect of drug treatment on body weight.* Repeated measures two-way ANOVA using the Greenhouse–Geisser correction indicated a significant interaction between day and treatment group on body weight ($F_{9.059,66.435} = 5.217$, $p < 0.05$), indicating that differences in weight gain occurred between treatment groups on different days. One-way ANOVAs followed by Fisher's LSD post-hocs showed that rats infused with the GOAT antisense gained less weight than those infused with vehicle or control infusates. These differences were statistically significant on days 11–17 of the treatment period

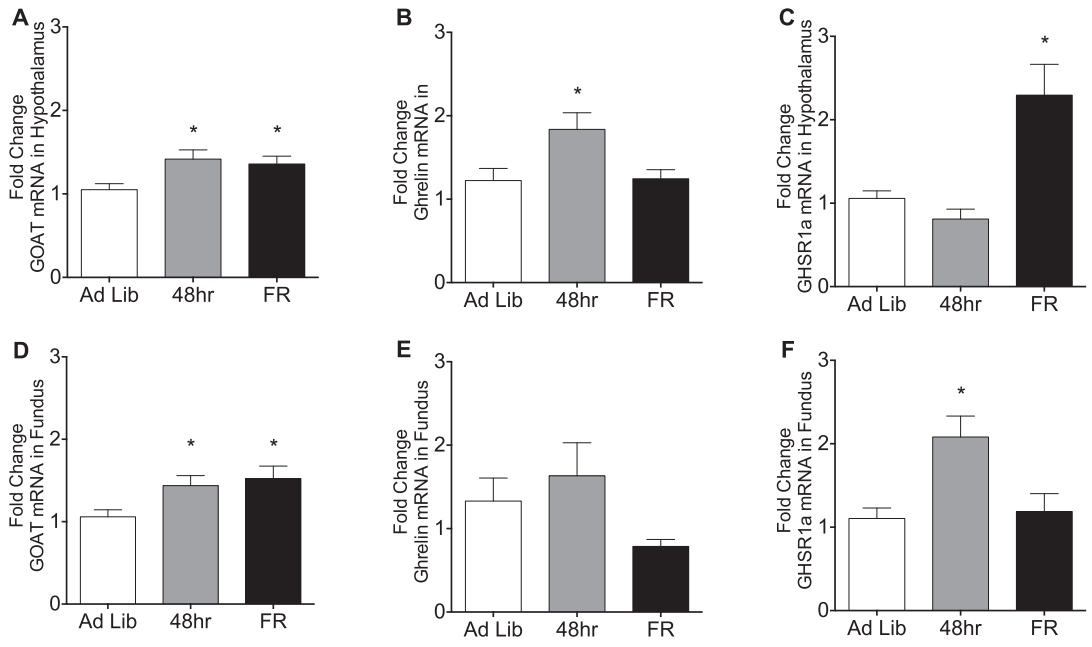


**Fig. 1.** qRT-PCR fold changes in the hypothalamus and fundus in response to food deprivation and food restriction. In the hypothalamus: fold changes in (A) GOAT, (B) ghrelin, and (C) GHSR1a mRNA. In the fundus: fold changes in (D) GOAT, (E) ghrelin, and (F) GHSR1a mRNA. Changes are in response to 48 h of food deprivation (48 h) or three weeks of food restriction (FR), relative to ad lib. animals (Ad Lib). All data are mean ± SEM. $^{*}p < 0.05$ when compared to ad lib. group; by one-way ANOVA with Fisher's LSD post hoc tests.

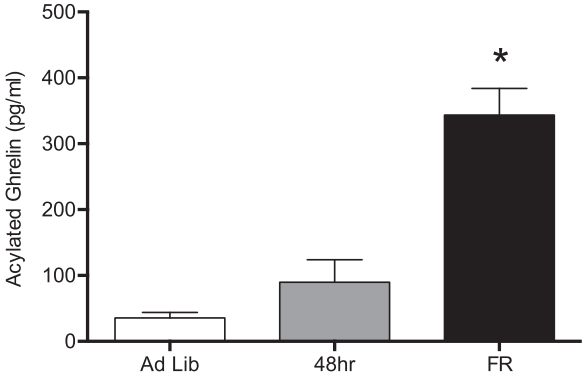


**Fig. 2.** Circulating acylated ghrelin levels. Plasma levels of active ghrelin obtained from an active ghrelin enzyme-linked immunosorbent assay (ELISA) in ad libitum (Ad Lib), 48-h food deprived (48 h), and three-week food restricted (FR) animals. All data are mean ± SEM. $^{*}p < 0.05$ when compared to both ad lib. and 48 h groups; by one-way ANOVA with Fisher's LSD post hoc tests.

($p < 0.05$). There were no differences between control groups (see Fig. 3A).

*Effect of drug treatment on food intake*

Repeated measures two-way ANOVA using the Greenhouse–Geisser correction indicated no significant interaction between day and treatment group on total food consumed over starting weight ($F_{3.442,25.241} = 0.988$, $p = 0.423$), indicating that no differences in food consumed over starting weight occurred between treatment groups on any day (Fig. 3B).

*Effect of drug treatment on weight gain as a function of food intake*

Repeated measures two-way ANOVA using the Greenhouse-Geisser correction indicated a significant interaction between day and treatment group ($F_{8.098,59.382} = 2.786$, $p < 0.05$), indicating that differences in total weight gain per total food consumed were present between treatment groups on different days. One-way ANOVAs followed by Fisher's LSD post-hocs showed that rats treated with the GOAT antisense gained less weight per food consumed on all days tested. This effect was statistically significant from experimental days 11–17 (Day 12 of treatment, $p = 0.068$). There were no differences between control groups (Fig. 3C).

## Discussion

The purpose of this experiment was two-fold: (1) to verify that GOAT mRNA expression exists in the rat hypothalamus and that the levels of these transcript are modulated by metabolic challenges, and (2) to examine the effect of central GOAT knockdown by vivo-morpholino anti-sense on food intake and weight gain in animals exposed to a high fat diet.

Data from Experiment 1 provide evidence that GOAT mRNA is modulated in the stomach and hypothalamus of rats following metabolic stress. These data supplement previous reports of the presence of GOAT message in the hypothalamus of rodents [8,22,24]. After 48 h of food deprivation, these increases in GOAT transcription occurred in concert with increases in hypothalamic ghrelin mRNA levels. While there is debate on whether ghrelin is synthesized within the hypothalamus [7,23,29], these data certainly support the notion that ghrelin is produced and acylated locally within the hypothalamus particularly in situations of negative energy state. Perhaps more interesting is data from Experiment 2. Here, knockdown of central GOAT mRNA expression using morpholino antisense oligonucleotides, decreased weight gain and caloric efficiency in rats exposed to a high fat diet. The decrease in weight gain seen in the current experiment is very similar to that seen in GOAT KO mice, or in mice that are treated with GO-CoA-Tat and exposed to diets rich in fatty acids [1,13]. In the current study, the lack of difference in caloric intake between knock-down and control rats suggest that the weight loss is not due to reductions in total caloric intake. Due to acylated ghrelin's important role in protecting fat stores [28,34], it is likely that GOAT knockdown animals show reduced body mass due to an increase in the utilization

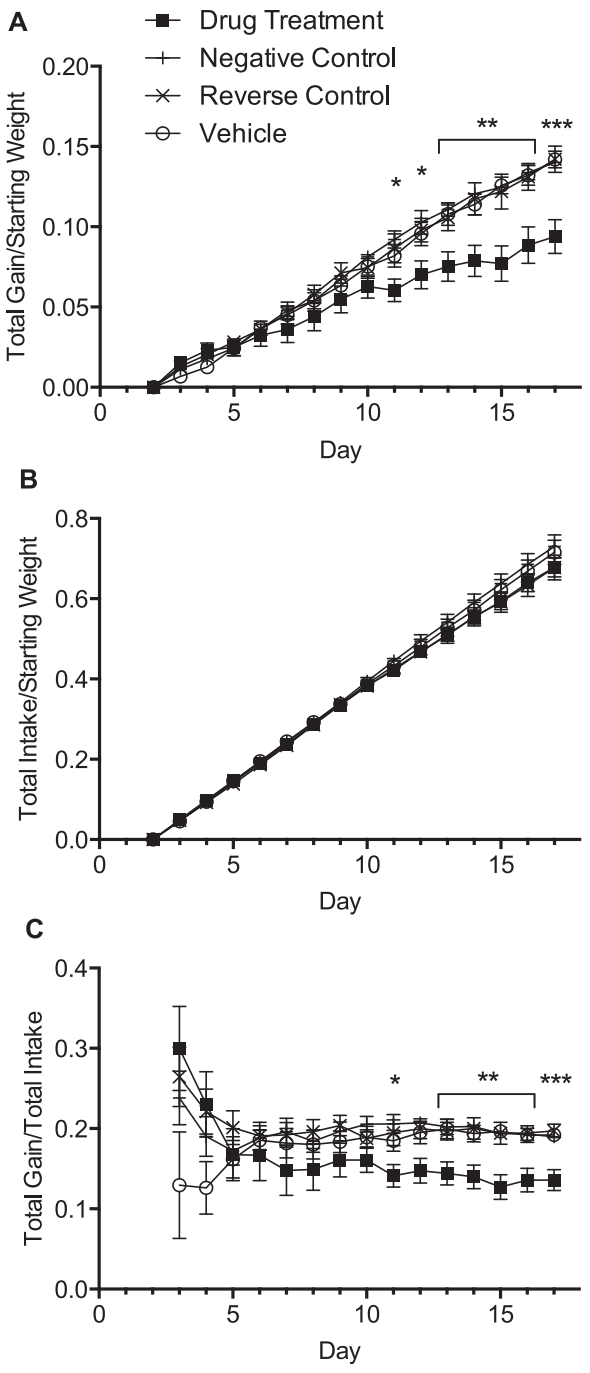


**Fig. 3.** Effects of knockdown of central GOAT by anti-sense administered to the lateral ventricles. Cannula implantation occurred on day 0, followed by a two-day recovery period. (A) VMO drug treatment effect on total percentage body weight gain since day 2 (two days after surgery). (B) VMO drug treatment effect on total HFD consumption since day 2, as a fraction of body weight on day 2. (C) VMO drug treatment effect on total body weight gain since day 2 per total HFD consumed since day 2. All data are mean ± SEM. $^{*}p < 0.05$ drug treatment vs. all control groups; $^{**}p < 0.01$ drug treatment vs. all control groups; $^{***}p < 0.001$ drug treatment vs. all control groups; by two-way repeated measures ANOVA, one-way ANOVA, and Fisher's LSD post hoc tests.

of body fat as a substrate for energy production. When adjusting weight gain per calorie consumed as a measure of metabolism, it is clear that animals treated with the GOAT antisense were gaining less weight per calorie consumed compared to rats in the control groups, again suggesting that central GOAT mRNA expression may play a role in energy balance. While it remains possible that central GOAT acylates ghrelin locally in the hypothalamus, it is also possible that this enzyme modifies other, yet to be determined, proteins that may in themselves be important modulators of food intake and energy balance in the hypothalamus. Supporting additional roles of GOAT beyond that of acylating ghrelin is Sakata and colleague's measurements showing that, of cells expressing GOAT mRNA, 14.5% of gastric mucosal cells and 19.4% of duodenal cells do not co-express ghrelin mRNA [24]. In spite of these results, it is important to note that knocking down gene expression with morpholino antisense sequences can cause toxic effects that could in themselves cause a decrease in weight gain. While preliminary data presented in this study shows that rats treated with non-selective morpholino sequences do not show a decrease in GOAT or NPY mRNA expression, nor show a decrease in weight gain, we cannot rule out that our antisense caused the decrease weight gain due to toxicity, so we remain cautious and suggest that further studies are conducted using specific GOAT inhibitors to supplement these data.

Given that the stomach fundus is believed to be the primary source of circulating ghrelin [4,19], it is interesting that we did not find any significant changes in ghrelin mRNA in samples of the fundus of rats fasted for 48 h or after three weeks of food restriction. This was not completely unexpected as others have found no changes in ghrelin mRNA expression in the stomach following a fast [18]. It is possible that food deprivation-induced elevations in plasma acylated ghrelin concentrations may be in fact due to increased GOAT mRNA expression, rather than increased ghrelin expression. This would suggest that a change in the acyl/desacyl ghrelin ratio occurs without changes in *denovo* ghrelin synthesis. It is also possible that other sources of circulating acylated ghrelin contribute to this increase including ghrelin secreted from other parts of the digestive tract. In addition to the changes in ghrelin and GOAT mRNA, elevations in GHSR1a mRNA were found after 48 h of food deprivation in the fundus and in response to three weeks of food restriction in the hypothalamus. The role of GHSR1a in the fundus has not been fully determined but the receptor may be an important component in signaling through the gastric vagal afferent pathway [5,6]. In addition, ghrelin also has autocrine effects in the stomach to modulate gut motility and to protect against gastric ulceration [14,26]. While it is difficult to speculate about the source of the differences in GHSR1a mRNA observed in the stomach versus the hypothalamus, it is quite possible that changes in GHSR mRNA expression in the fundus are not associated with energy state, but more with gastro protection during the acute fast, and the result of compensatory down-regulation of GHSR1a mRNA expression following prolonged elevations in circulating acylated ghrelin in the chronic food restriction paradigm.

Our results indicating that acylated ghrelin levels are highest with long-term metabolic challenge suggest that the ghrelin-GOAT system may be particularly important for circumstances where undernutrition is prolonged. Indeed, ghrelin has been implicated in the mechanisms through which mammals adapt to undernutrition including the generation of glucose. Interestingly, both GOAT and GHSR KO mice have difficulty adapting to caloric restriction paradigms and are hypoglycemic, and this state can be reversed in GOAT KO animals by injections of ghrelin or growth hormone [2,33].

In all, our data suggest that the GOAT enzyme is produced in the hypothalamus and it plays a role in regulating energy balance. The mechanisms by which GOAT has these effects remain unclear. One could suggest that these mechanisms involve the acylation of locally produced ghrelin. Alternatively, GOAT may generate these effects independently from ghrelin, yet work is needed to determine if this is the case.

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