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VGF-Derived Peptide, TLQP-21, Regulates Food Intake and Body Weight in Siberian Hamsters

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The Siberian hamster survives winter by decreasing food intake and catabolizing abdominal fat reserves, resulting in a sustained, profound loss of body weight. VGF gene expression is photoperiodically regulated in the hypothalamus with significantly higher expression in lean Siberian hamsters. The aim of this study was to investigate the role of VGF in regulating these seasonal cycles by determining the effects of a VGF-derived peptide (TLQP-21) on food intake and body weight. Acute intracerebroventricular administration of TLQP-21 decreased food intake, and chronic treatment caused a sustained reduction in food intake and body weight and decreased abdominal fat depots. Behavioral analysis revealed that TLQP-21 reduced meal size but not the frequency of feeding bouts, suggesting a primary action on satiety. Hamsters treated with TLQP-21 lost a similar amount of weight as a pair-fed group in which food intake was matched to that of the TLQP-21-treated group. Central or peripheral treatment with TLQP-21 did not produce a significant effect on resting metabolic rate. We conclude that the primary action of TLQP-21 is to decrease food intake rather than increase energy expenditure. TLQP-21 treatment caused a decrease in UCP-1 mRNA in brown adipose tissue, but hypothalamic expression of orexigenic and anorexigenic neuropeptide genes remained unchanged after TLQP-21 treatment, although compensatory increases in NPY and AgRP mRNA were observed in the pair-fed hamsters. The effects of TLQP-21 administration are similar to those in hamsters in short days, suggesting that increased VGF activity may contribute to the hypophagia that underlies the seasonal catabolic state. (*Endocrinology* 148: 4044–4055, 2007)

PRO-VGF PROTEIN IS the primary 68-kDa product of the *vgf* gene (1). VGF mRNA is synthesized widely and found to be abundantly expressed in neurons in the hippocampus, cerebral cortex, amygdala, midbrain and thalamus (2–6). The VGF protein is selectively processed into peptides in a tissue-specific manner in neuroendocrine and neuronal cells and released through a regulated secretory pathway (7–9). The highest concentrations of VGF immunoreactivity have been found in the medial hypothalamus, particularly in the arcuate nucleus (Arc), the paraventricular nucleus, the supraoptic nucleus, and in the suprachiasmatic nucleus (3, 4). VGF-containing neurons in these nuclei project both to the median eminence and to the posterior pituitary (10); thus, hypothalamic VGF or VGF-derived peptides may have central actions as well as peripheral functions after reaching target tissues via the circulation.

Recent evidence suggests an important role for VGF and/or one of its processed peptides in the regulation of energy balance. Fasting increases VGF mRNA expression in the Arc of mice (11), and VGF-null mice have a distinct phenotype. They are thin, small, hypermetabolic, hyperactive, and infertile, with markedly reduced leptin levels and fat stores (12). They also have altered hypothalamic proopiomelanocortin (POMC), neuropeptide Y (NPY), and agouti gene-related peptide (AgRP) expression in the Arc (12). However, the direction of change in expression of these genes (increased NPY and AgRP, decreased POMC) would suggest that they are compensatory responses reflecting the establishment of the catabolic state, rather than being causative in generating the catabolism.

The Siberian hamster (*Phodopus sungorus*) is being used increasingly as a rodent model for understanding the long-term central control of caloric intake and expenditure because it expresses profound seasonal cycles of energy metabolism as an adaptation to survive winter (13–17). Exposure to short photoperiods causes a 25–35% reduction in body weight during winter even under conditions of *ad libitum* food availability (16, 18, 19). This decrease in body weight results from decreased appetite and the catabolism of intraabdominal fat stores and is entirely programmed by a decrease in photoperiod (20). This catabolic state is associated with a decrease in both peripheral leptin concentrations (21) and in leptin mRNA levels in white adipose tissue (22, 23). Our overall aim is to understand the central mechanisms that enable the hamsters to decrease their ip fat reserves and therefore leptin levels but not engage in compensatory hyperphagia. We have recently shown that the gene encoding

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Abbreviations: AgRP, Agouti gene-related peptide; Arc, arcuate nucleus; CART, cocaine- and amphetamine-regulated transcript; icv, intracerebroventricular; NPY, neuropeptide Y; NS, not significant; POMC, proopimelanocortin; SSC, standard saline citrate; UCP-1, uncoupling protein 1.

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VGF mRNA expression is photoperiodically regulated with significantly higher expression in winter in the dorsomedial posterior Arc (24). After switching from an inhibitory short photoperiod to a stimulatory long photoperiod, *VGF* expression decreases rapidly, ahead of body weight recovery (24). Thus, we hypothesize that increased VGF production may underlie the development of aspects of the winter catabolic state observed in Siberian hamsters.

Native VGF is a large protein (617 amino acids) that is cleaved by prohormone convertases into a number of biologically active peptides (9). TLQP-21 has recently been reported to be one of the major peptide fragments present in the brain of rats (25) and has been shown to exert catabolic effects on energy metabolism in mice (25), contrary to the predictions that might be made based on observations in the VGF-global knockout mouse. In this study we have investigated the acute and chronic action of VGF on food intake, body weight, energy metabolism, and hypothalamic gene expression in the Siberian hamster using the VGF-derived peptide TLQP-21 as an experimental tool.

Materials and Methods

Animals

Experimental animals were obtained from a colony of Siberian hamsters (*Phodopus sungorus*), maintained at the University of Nottingham Medical School (26). They were housed in individual cages under controlled temperature (21 ± 1 C) and light (16-h light, 8-h dark cycle; lights off at 1100 h). Another group of Siberian hamsters were individually housed and maintained at University of Nottingham, Sutton Bonington, under controlled temperature (21 ± 1 C) and light (16-h light, 8-h dark cycle, lights off at 1700 h). All animals had *ad libitum* access to food intake and water, unless stated otherwise. All animal procedures were approved by the University of Nottingham Local Ethical Review Committee and were carried out in accordance with the Animals Scientific Procedures Act (UK) 1986 (project license PPL 40/2372).

Peptides

TLQP-21 and the scrambled peptide (consisting of the same amino acid sequence as TLQP-21 but in a different order) were synthesized on an Applied Biosystems (Foster City, CA) model 433 synthesizer using FastMoc protocols, according to manufacturer's recommendations. All synthesis reagents were from Applied Biosystems or Novabiochem (Nottingham, UK). 5-Carboxyfluorescein was coupled to the N terminus of the resin-bound peptide with no changes to the synthesis protocol. Cleavage and deprotection reactions were performed in trifluoroacetic acid (Applied Bioscience, Foster City, CA)/tri-isopropyl silane (Sigma, Dorset, UK)/water (Fischer Biosciences, Loughborough, UK) in a ratio of 95:2.5:2.5 (vol/vol) at room temperature for 2 h.

Intracerebroventricular (icv) cannulation and infusion

Animal surgical procedures and handling were carried out as previously described (13, 14, 27). Hamsters were anesthetized with a mixture of ketamine (Vetalar 100 mg/kg ip; Forte Dodge Animal Health Ltd., Southampton, UK) and medetomidine (Dormitor 1 mg/kg ip; Pfizer Ltd., Kent, UK) in a ratio of 1:4. Analgesia was maintained via sc injection of carprofen (50 mg/kg Rimadyl; Pfizer) and administered before surgery. Animals were placed in a Kopf stereotaxic frame (David Kopf Instruments, Melville, NY) with the incisor bar positioned level with the interaural line, and a permanent 22-gauge guide cannula (Bilaney Consultants, Kent, UK) was stereotaxically implanted into the third ventricle 6.5 mm below the surface of the dura after deflection of the superior midsagittal sinus as previously described (13, 14, 27). Two stainless steel screws were inserted into the cranium, and the cannula was fixed to these with dental cement. An obturator (Bilaney Consultants) was inserted into the guide cannula to maintain patency. After surgery, the animals were treated with atipamezole to reverse the anesthesia (Antisedan 1 mg/kg sc; Pfizer) and fluid replacement (0.9% saline). The surgically prepared hamsters were allowed a 7-d recovery period after which they were handled on a daily basis and were habituated to the experimental process by a control vehicle infusion (1 μl 0.9% saline) before the onset of the study. All infusions (1 μl total volume) were carried out in conscious, free-moving hamsters at a rate of 1 μl/min, using a 29-gauge stainless steel injector placed in and projecting 1 mm below the tip of the guide cannula. The injector was left in place for 3 min to allow diffusion of the injected solution. The cannula stylet was replaced immediately after withdrawal of the infusion cannula.

Palatable diet

Before injection, lab chow was removed from the hopper and replaced by a preweighed amount of a palatable diet (moist lab chow) inside the home cage. To estimate the reduction in weight of the test meal through water evaporation, three preweighed dishes also containing wet pellets were placed in control cages alongside the experimental hamster cages. The calculation of the food intake at any given time by each hamster included the deduction of the average evaporation of water of the three control meals.

Oxygen consumption (VO_2)

Oxygen consumption (VO_2) in experiments 1, 3, and 4 was determined in a closed-circuit calorimeter maintained at thermoneutral temperature (29 C) as previously described (28). The system consists of eight calorimetric chambers in which Siberian hamsters are studied individually. Carbon dioxide and water are removed from the system using soda lime and silica gel, respectively, and VO_2 for each chamber calibrated to relative change in pressure. VO_2 was measured every 8 min and expressed as ml O_2 per minute per kilogram metabolic body weight ($ml/min \cdot kg^{0.75}$). For each hamster, VO_2 was monitored for 88 min before injection (baseline) and another 128 min after injection. All animals were acclimatized to the calorimeters and procedures by measuring basal VO_2 on at least one occasion before investigating the effects of the test compounds. Experimental treatments were given to hamsters during the light phase, at approximately 1100 h, 88 min after being placed into the calorimeter. The first 24 min were discarded to allow for a settling period.

Metabolic gases and consummatory behavior (experiment 2)

Multiple parameters including VO_2, CO_2 production (VCO_2), and various parameters of eating behavior including frequency and duration of feeding bouts, food consumption per bout and total food intake, and locomotor activity, were measured using a Comprehensive Lab Animal Monitoring System (Linton Instrumentation, Linton, UK, and Columbus Instruments, Columbus, OH). This is a modified open-circuit calorimeter, and the configuration used for Siberian hamsters consisted of eight mouse chambers in which hamsters were studied individually. The chambers had feeders positioned in the middle of the cages, and all removal of food was recorded. A bout of food intake (meal) was defined as an intake of greater than 0.02 g. We operated the system with an air intake of 0.6 liters/min per chamber, and an extracted outflow of 0.4 liters/min. Water was provided by dropper bottles, but water intake was not recorded. Activity was recorded when two or more consecutive infrared beams positioned approximately 2 cm apart were broken. All measurements were taken at an ambient temperature of 21–22 C.

Experiment 1: effect of acute icv administration of TLQP-21 on food intake, body weight, behavior, and resting metabolic rate

Male Siberian hamsters received a single infusion of either saline or VGF-derived peptide TLQP-21 (1, 5, or 25 μg) in a pseudorandom order so that each hamster served as its own control over the course of 4 wk. Infusions were carried out shortly before lights out (1100 h). After the infusion, hamsters were returned to their home cages and offered a preweighed amount of the palatable diet. Food was reweighed at 1, 2,

4, 6, and 24 h after injection, whereas body weight and water intake were measured 24 h after injection using a Fisher Scientific FP-300 series balance (Fisher Scientific Ltd., Leicester, UK). On each experimental occasion, the behavior of each hamster was monitored in its home cage for 5 sec in every minute for 1 h after lights off, as previously described (13, 14, 29). Five behavioral categories whose definitions are based on Halford *et al.* (30) were used in this study: feeding (hamster observed to be investigating/eating the test meal of palatable chow), drinking (hamster at/drinking from water bottle), grooming (hamster grooming), locomotor (ambulatory, climbing on cage bars, burrowing, or rearing), and inactive (hamster at rest/sleeping).

Oxygen consumption (VO_2) was also monitored in a separate group of male Siberian hamsters (n = 4 per treatment) that received a single icv infusion of vehicle or TLQP-21 (5 or 25 μg) during the light phase. VO_2 was measured for an 88-min baseline period, then for another 128 min after infusion.

Experiment 2: effects of acute icv administration of TLQP-21 on patterns of meals, activity, and oxygen consumption

On two occasions, eight adult male Siberian hamsters that had previously been habituated to a powdered lab chow diet were placed into individual Comprehensive Lab Animal Monitoring System chambers 12 h before infusion to allow habituation to the novel environment. They had *ad libitum* access to ground powdered normal chow and water throughout the test period. The hamsters received a single infusion of either saline or VGF-derived peptide TLQP-21 (5 μg) in a pseudorandom order so that each hamster served as its own control over the course of 2 wk. Infusions were carried out shortly before lights out (1100 h). Food intake and activity were monitored for 24 h after the infusion over 9-min epochs, and meal sizes were noted every time an animal consumed more than 0.02 g. VO_2 and VCO_2 were monitored over 9-min epochs for a total of 24 h after treatment. All data were collected using the OxyMax Windows software version 4.0 (Columbus Instruments).

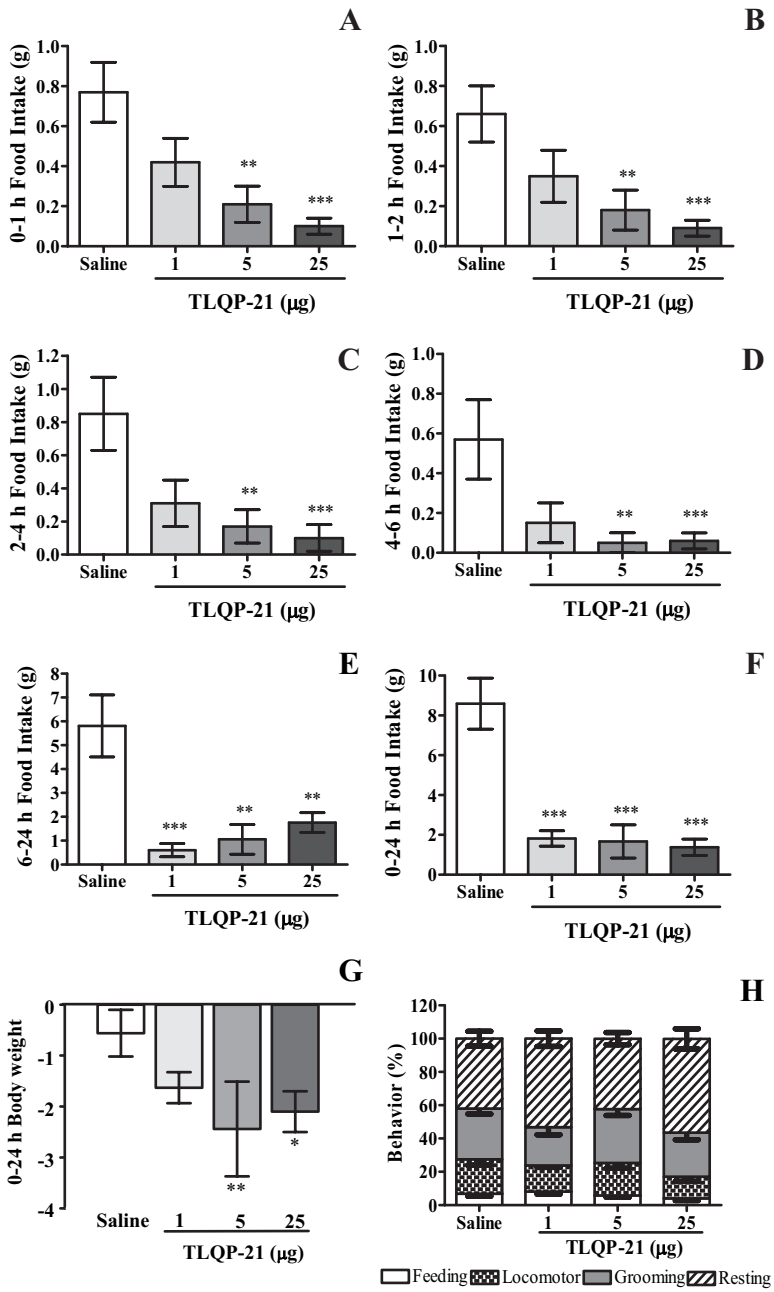

FIG. 1. Experiment 1: effects of acute icv administration of TLQP-21 on food intake, body weight, and behavior. *Ad libitum*-fed male Siberian hamsters received a single 1-μl icv infusion of either saline or VGF-derived peptide TLQP-21 (1, 5, or 25 μg) in a pseudorandom order so that each animal acted as its own control. Food intake was measured 0–1 h (A), 1–2 h (B), 2–4 h (C), 4–6 h (D), 6–24 h (E), and 0–24 h (F) after injection. Change in body weight (G) was measured 24 h after injection. Behavior (H) was observed 0–1 h after injection. *Bars* (expressed as mean ± SEM) depict the proportion of observations of each of the predefined behaviors. Significance values are as indicated: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ *vs.* saline (n = 11 per group).

Experiment 3: effect of chronic icv administration of TLQP-21 on food intake, body weight, oxygen consumption, organ weight, and hypothalamic gene expression

Male Siberian hamsters were randomized by weight into four groups (n = 5–8 per group): 1) vehicle-treated (icv saline) with *ad libitum* access to normal lab chow, 2) TLQP-21-treated (icv TLQP-21, 5 μg) with *ad libitum* access to normal lab chow, 3) control (icv scrambled peptide, 5 μg) with *ad libitum* access to normal lab chow, and 4) pair-fed (icv saline and food restricted to the median intake of the TLQP-21-treated group as measured over the previous 24 h). Hamsters received daily 1-μl icv infusions shortly before lights out (1100 h) for seven consecutive days (d 0 = injection 1). Food intake, body weight, and water intake were monitored daily. On d 7 (or d 8 for the pair-fed group), the hamsters were euthanized with sodium pentobarbitone (Euthatal; Rhone Merieux, Harlow, UK), and their brains were removed and snap frozen in dry ice for subsequent measurement of hypothalamic peptide mRNA expression by *in situ* hybridization [AgRP, NPY, POMC, and cocaine- and amphetamine-regulated transcript CART)]. A biopsy of the brown adipose tissue was snap frozen in liquid nitrogen for subsequent analysis of uncoupling protein 1 (UCP-1) mRNA expression. Epididymal white adipose tissue pads, the pituitary gland, adrenal glands and testes were removed and weighed.

Oxygen consumption (VO_2) was also monitored in a separate group (n = 4) of male Siberian hamsters that received a similar daily TLQP-21 treatment. Hamsters received an initial icv infusion of TLQP-21 (5 μg) in the light phase after an 88-min baseline period. VO_2 was measured for another 128 min after injection. These hamsters then received subsequent daily infusions of TLQP-21 (5 μg) for another six consecutive days, and VO_2 was measured on d 1, 3, and 6 for 88 min before each infusion and for 128 min afterward.

UCP-1 levels. Total RNA was extracted with Trizol using the recommended protocol for tissue high in lipid content (Invitrogen, Paisley, UK). After the isopropanol precipitation step, extracted RNA was resuspended in 50 μl RNase-free water. Quantification and RNA integrity was assessed by Agilent RNA lab-on-a-chip analysis. A volume containing 2 μg total RNA was dispensed into microfuge tubes, and the volume of the RNA solution reduced to 5 μl by vacuum concentration.

Before electrophoresis, 3.5 μl formaldehyde, 10 μl formamide, and 1 μl 10× MOPS buffer [1× MOPS buffer contains 20 mM 3-(*N*-mopholino)propanesulfonic acid, 2 mM sodium acetate, and 1 mM EDTA, pH 8.0] was added to each RNA sample. RNA was heated to 65 C for 5 min, cooled on ice, and loaded onto a 1% formaldehyde agarose gel. Electrophoresis was performed at 100 V for 2 h. RNA was visualized by ethidium bromide staining. The gel was soaked in 0.15 M NaCl, 50 mM NaOH for 30 min to nick and denature the RNA. The gel was neutralized by soaking in 0.15 M NaCl, 50 mM Tris-HCl (pH 7) for 30 min. RNA was transferred to Genescreen nylon membrane (PerkinElmer Life Sciences, Bucksinghamshire, UK) using a posiblotter (Stratagene Europe, Amsterdam, The Netherlands) and UV cross-linked.

The RNA blot was hybridized with a PCR fragment for UCP-1 amplified from brown adipose tissue with the following primers based on the UCP-1 sequence of Siberian hamster (GenBank AF271263 amplifying between bases 226 and 904): forward primer, 5′-AGTGGTCTGCCCGCTGGTAT; reverse primer, 5′-TCTGCCTCGACTTCATCAACTCTT. Conditions were 94 C for 1 min, 94 C for 30 sec, 60 C for 30 sec, and 72 C for 45 sec for 35 cycles and a final incubation at 72 C for 10 min. The amplified DNA fragment was confirmed to be the UCP-1 sequence by DNA sequencing. The DNA fragment was labeled with [^{32}P]dCTP using the High Prime labeling kit (Roche Applied Science, Lewes, East Sussex, UK) in the presence of 1.85 MBq [^{32}P]dCTP (MP Biomedicals, Irvine, CA). Labeled DNA was separated from unincorporated nucleotides with a probe-quant spin column (GE Healthcare, Amersham, Buckinghamshire, UK). Hybridization was performed at 60 C for 1 h in QuickHyb solution (Strategene Europe). The filter was washed twice at room temperature in 2× standard saline citrate (SSC), 0.1% SDS (1× SSC is 0.15 M NaCl, 15 mM sodium citrate) for 15 min and once in 0.2× SSC, 0.1% SDS for 30 min. Equal loading was ascertained with an 18S rRNA probe (31) hybridizing at 50 C in QuickHyb solution and washing the filter twice with 2× SSC, 0.1% SDS at room temperature, 5 min per wash, and 0.5% SSC, 0.1% SDS at 50 C for 15 min.

The filter was apposed to Kodak Biomax autoradiographic film. Quantification was performed by image analysis using Image-Pro PLUS version 4.1.0.0 analysis software (Media Cybernectics, Wokingham, UK).

Hypothalamic gene expression. DNA sequences and riboprobe generation for *in situ* hybridization for NPY, AGRP, POMC, and CART have been previously described (32). *In situ* hybridization using ^{35}S-labeled riboprobes was performed as described previously (32). Probes were hybridized at 58 C for 16 h. Tissue sections received a final wash in 0.1× SSC at 60 C. Sections were apposed to Kodak Biomax film for 7 d. Autoradiographic films were scanned at 600 dpi on a Umax scanner linked to a PC running Image-Pro PLUS version 4.1.0.0 analysis software, and the area and density of the signal overlying the Arc was determined for each probe.

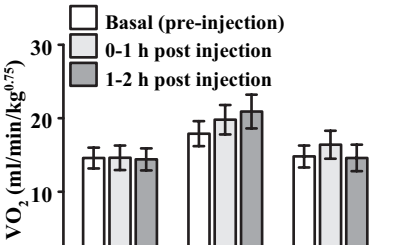

FIG. 2. Experiment 1: effect of acute icv administration of TLQP-21 on oxygen consumption. *Ad libitum*-fed male Siberian hamsters received a single icv infusion of either saline or VGF-derived peptide TLQP-21 (5 or 25 μg) in a pseudorandom order. Oxygen consumption (VO_2) was measured in closed-circuit calorimeters over 8-min epochs for 88 min before injection and for another 128 min afterward. Values are group means ± SEM for the last hour before treatment (basal) and for 0–1 h and 1–2 h after injection (n = 4 per group).

Experiment 4: effect of acute systemic (ip) administration of TLQP-21 on food intake, body weight, behavior, and oxygen consumption

Male Siberian hamsters received a single ip injection (10 μl/g body weight) before lights out (1100 h) of either saline or TLQP-21 (1, 5, or 25 mg/kg) in a pseudorandom order so that each hamster served as its own control over the course of 4 wk. After the injections, animals were returned to their home cages containing a preweighed amount of the palatable diet (moist lab chow). Food was reweighed at 1, 2, 4, 6, and 24 h after injection, and body weight and water intake were measured 24 h after injection. Behavior was also monitored over the first hour after treatment as described above. Oxygen consumption (VO_2) was also monitored in a separate group (n = 8–9 per treatment) of male Siberian hamsters that received a single ip injection in the light phase of vehicle or TLQP-21 (1, 5, or 25 mg/kg). VO_2 was measured for an 88-min baseline period and then for another 128 min after treatment.

Statistical analyses

Data are presented as mean ± SEM. For the acute studies (experiments 1 and 4), data for food intake and body weight were compared between groups by one-way ANOVA followed by a *post hoc* Bonferroni test (SigmaStat 2.03, Chicago, IL). Data from the behavioral observations were compared using the nonparametric Kruskal-Wallis test (SigmaStat 2.03). For the chronic study (experiment 2), data for food intake and body weight were compared by a repeated-measure one-way ANOVA followed by a *post hoc* Bonferroni test (Systat, Chicago, IL), whereas organ weight and gene expression between groups were compared by one-way ANOVA followed by a *post hoc* Bonferroni test (Systat). In the indirect calorimetry studies (experiments 1 and 4, the raw VO_2 data for each hamster in 8-min epochs were initially averaged to generate a mean value for the last hour before treatment (basal) and for the first and second hours after treatment. These individual mean values were com-

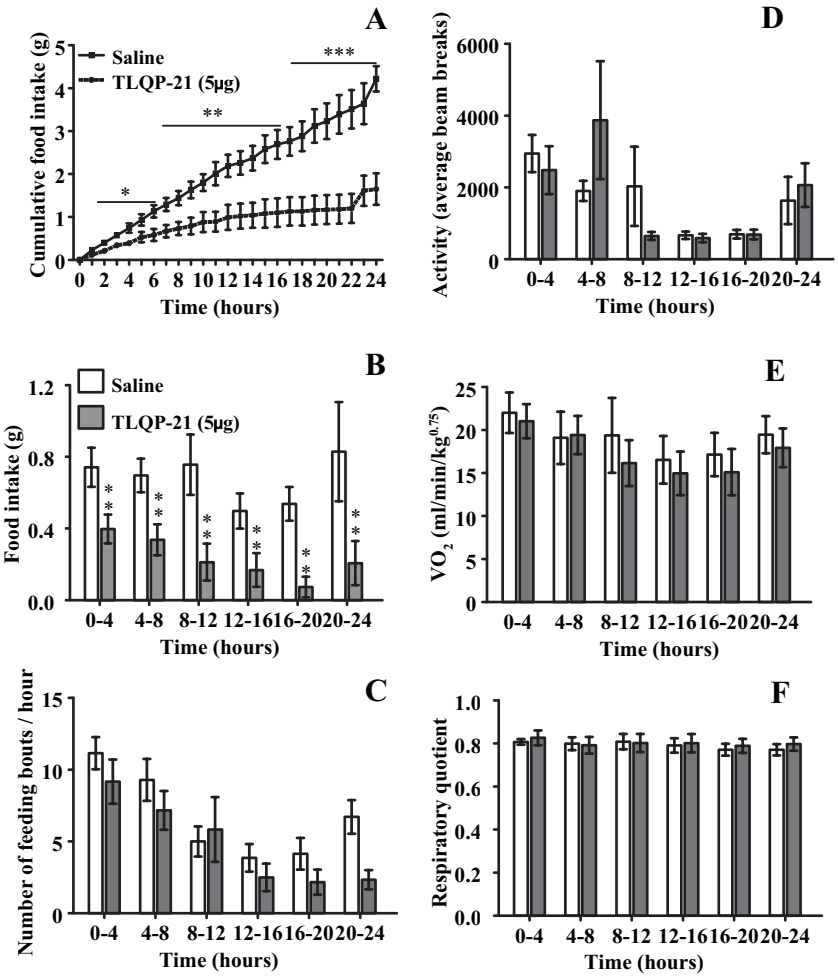

FIG. 3. Experiment 2: effects of acute icv administration on patterns of meals, activity, and oxygen consumption. *Ad libitum*-fed male Siberian hamsters received a single icv infusion of either saline or VGF-derived peptide TLQP-21 (5 μg) in a pseudorandom order. Metabolic and food intake parameters were measured using an OxyMax open-circuit calorimeter. Cumulative and interval food intake (A and B) and the number of feeding bouts (C), defined as an intake greater than 0.02 g, were measured over 9-min epochs. Ambulatory activity (D) of each animal was measured simultaneously using optical beams. Oxygen consumption (VO_2) (E) and respiratory quotient (F) were also measured over 9-min epochs. Values are group means ± SEM (n = 5 per group), and significance values are as indicated: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ *vs.* saline.

pared in using a two-way ANOVA [effect of time (repeated measure) *vs.* effect of treatment] (Prism 4.0; GraphPad, San Diego, CA). In experiment 2, the values were compared using two-way repeated-measure ANOVA (Prism 4.0). In all cases, $P < 0.05$ was considered statistically significant.

Results

Experiment 1: effect of acute icv administration of TLQP-21 on food intake, body weight, behavior, and oxygen consumption

The icv administration of TLQP-21 at the onset of the dark phase dose-dependently decreased food intake (Fig. 1, A–F). At the higher doses of TLQP-21, this effect occurred within 1 h after infusion compared with saline-treated hamsters ($P < 0.001$ *vs.* saline, respectively; Fig. 1A) and persisted for up to 24 h (Fig. 1, B–E). A significant suppressive effect of the lowest dose was also detected when data for the entire 24 h after treatment period were summated (all doses $P < 0.001$ *vs.* saline; Fig. 1, E and F). The icv administration of TLQP-21 (5 and 25 μg) significantly decreased body weight 24 h after injection compared with saline-treated animals (Fig. 1G).

The number of observations made for each defined behavior was calculated and expressed as a percentage of the total number of observations in 1 h after injection of VGF or saline into the third ventricle. No sedation or hyperactivity was observed after icv administration of VGF (1, 5, or 25 μg) when compared with saline-treated (Fig. 1H). Despite the reduction in food intake in the first hour after 5 or 25 μg VGF treatment, there was no significant difference in the proportion of time that the hamsters were observed to be investigating or eating the test meal (Fig. 1H). There were no adverse behaviors observed in any treatment groups.

The mean baseline VO_2 in male Siberian hamsters in the light phase varied between 14.1 ± 0.3 and 17.2 ± 0.6 ml/min·$kg^{0.75}$ (Fig. 2). Although there was a small trend toward slightly elevated values after the 5- and 25-μg TLQP-21 icv treatments (Fig. 2), two-way ANOVA revealed that this was not significant [treatment *vs.* time interaction, F = 2.21; P = not significant (NS)].

Experiment 2: effects of acute icv administration of TLQP-21 on patterns of meals, activity and oxygen consumption

The icv administration of TLQP-21 (5 μg) at the onset of the dark phase decreased food intake. A significant effect was apparent from the second hour after treatment (Fig. 3A) and persisted throughout the 24-h period (Fig. 3B; effect of treatment F = 18.2; $P < 0.001$ but no significant treatment *vs.* time interaction). To investigate whether hamsters were experiencing nausea, malaise, or other behavioral effects that could impact upon ingestive behavior, we examined the frequency of feeding bouts and also the locomotor activity of the hamsters throughout the 24-h period. With the exception of one

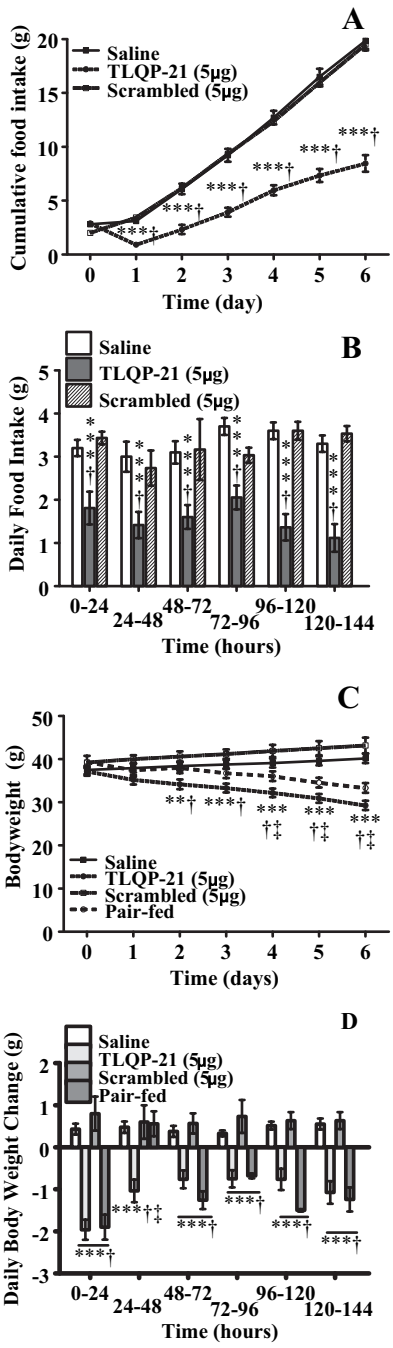

FIG. 4. Experiment 3: effects of chronic icv administration of TLQP-21 on food intake, body weight, and behavior. Cumulative and interval food intake (A and B) and body weight (C and D) in male Siberian hamsters fed *ad libitum* and treated daily before lights out (1100 h) with 1) 1 μl icv vehicle (saline), 2) 5 μg TLQP-21 in 1 μl, or 3) 1 μl scrambled peptide (5 μg). In a fourth group, hamsters (pair-fed) were treated daily with 1 μl icv saline, but their food intake was restricted to the median food intake of the TLQP-21-treated group. Values are group means ± SEM (n = 8–11 per group), and significance values are indicated: *, $P < 0.05$; **, $P < 0.01$; *** $P < 0.001$ *vs.* saline; †, $P < 0.001$ *vs.* scrambled peptide; ‡, $P < 0.05$ *vs.* pair-fed.

period 20–24 h after treatment, there was no significant effect of TLQP-21 treatment on frequency of meals (Fig. 3C, time *vs.* treatment interaction F = 1.4, P = NS), although there was, as expected, a significant effect of time, with frequency of meals higher in the dark phase in both groups than in the light phase (F = 16.1; $P < 0.0001$). There was no significant difference in activity between saline-treated and TLQP-21-treated hamsters in either the dark or light phase (Fig. 3D, time *vs.* treatment interaction F = 1.5; P = NS), although there was a significant decrease in activity in the light phase (Fig. 3D, effect of time F = 4.7; $P < 0.01$). There was no significant effect of treatment on VO_2 (Fig. 3E), but there was a significant effect of time (F = 4.8; $P < 0.005$), indicating that VO_2 decreased during the light phase in both groups. There were no significant effects of either treatment or time on respiratory quotient (Fig. 3F).

Experiment 3: effect of chronic icv administration of TLQP-21 on food intake, body weight, oxygen consumption, organ weight, and hypothalamic gene expression

Daily food intake was lower in hamsters treated daily with TLQP-21, and by d 6, food intake was decreased by 56% compared with saline-treated ($P < 0.001$ *vs.* saline; Fig. 4A). The hamsters treated with TLQP-21 lost body weight compared with those treated with saline (Fig. 4C). Analysis of daily food intake and body weight revealed that the response to TLQP-21 was maintained throughout the treatment period (Fig. 4, B and D). Treatment of hamsters with a scrambled peptide had no effect on food intake or body weight when compared with saline-treated, however these parameters were significantly different to the TLQP-21-treated hamsters ($P < 0.001$ *vs.* scrambled; Fig. 4, A and C). To investigate whether the effect of central TLQP-21 treatment on body weight might be due to the decrease in food intake or to increased energy metabolism, a group of hamsters were restricted to the food intake of the TLQP-21-treated hamsters (pair-fed). The rate of weight loss in the pair-fed hamsters was similar to that of the TLQP-21-treated group (Fig. 4C), and throughout the treatment period, similar degrees of weight loss were seen when comparing the TLQP-21-treated and pair-fed groups (Fig. 4D). Daily infusions of TLQP-21 had no significant effect on oxygen consumption at any time point when compared with baseline (effect of time F = 1.168; P = NS) (Fig. 5, A–E).

Organ weights. The decrease in body weight after TLQP-21 treatment was reflected by a reduction in adiposity. Epididymal white adipose tissue weights at d 6 were significantly decreased after TLQP-21 treatment ($P < 0.001$ *vs.* saline; $P < 0.01$ *vs.* scrambled and pair-fed; Fig. 6A). TLQP-21 treatment also caused a small but significant decrease in the paired testes weight ($P < 0.05$ *vs.* saline; Fig. 6B), but there were no significant effects of TLQP-21 treatment on the weights of the pituitary (Fig. 6C) or adrenal glands (Fig. 6D).

Hypothalamic gene expression. Expression of NPY, AgRP, POMC, and CART in the Arc was unaffected by the 7-d treatment with TLQP-21 (Fig. 7). However, there were significant increases in the pair-fed control hamsters in the expression of NPY ($P < 0.005$ *vs.* saline; Fig. 7A), AgRP ($P < 0.05$ *vs.* saline; Fig. 7B), and CART ($P < 0.05$ *vs.* saline; Fig. 7C). There was no significant difference in POMC expression between any of the experimental groups (Fig. 7D).

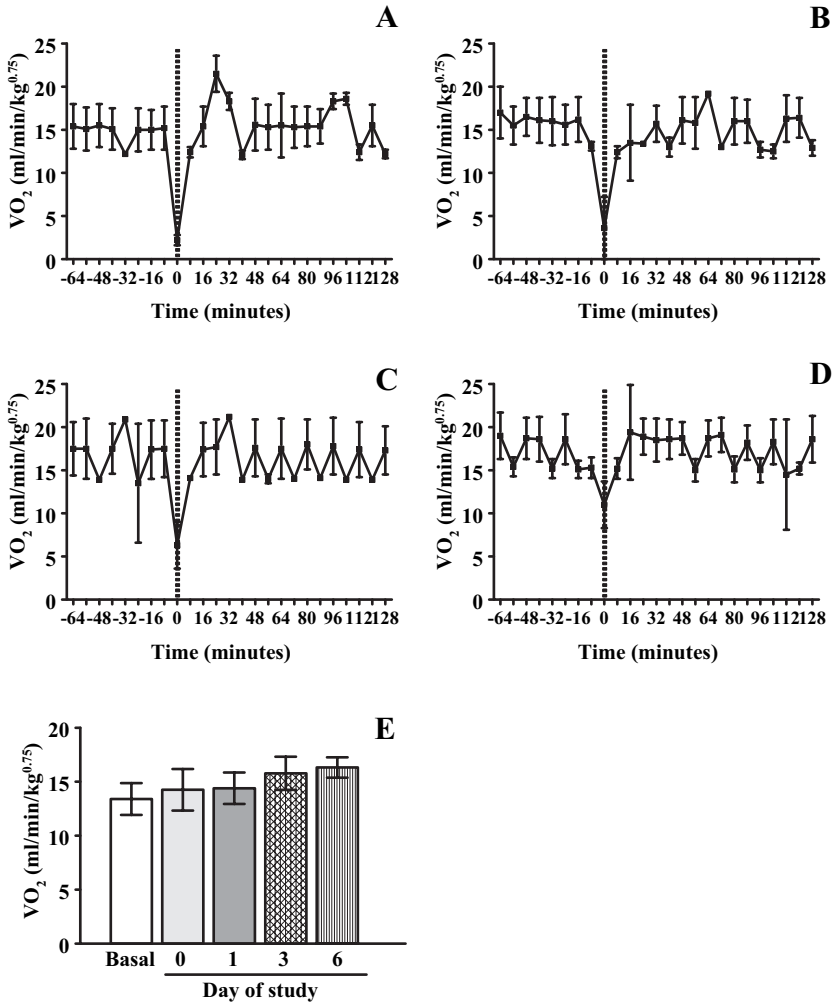

FIG. 5. Experiment 3: effect of chronic icv administration of TLQP-21 on oxygen consumption. *Ad libitum*-fed male Siberian hamsters received a daily 1-μl icv infusion of TLQP-21 (5 μg) for 7 d (d 0–6). VO_2 was monitored for 1 h before each infusion and for an additional 128 min after infusion in 8-min epochs in closed-circuit calorimeters on d 0 (A), 1 (B), 3 (C), and 6 (D). Values are expressed as group means ± SEM (n = 4 per group). VO_2 was calculated for a 1-h period before the first infusion and for the first hour after treatment on d 0, 1, 3, and 6 (E).

UCP-1 expression. UCP-1 mRNA expression in brown adipose tissue was significantly decreased in both the TLQP-21-treated hamsters and the pair-fed hamsters ($P < 0.005$ *vs.* saline and $P < 0.001$ *vs.* saline, respectively; Fig. 7E).

Experiment 4: effect of acute systemic administration of TLQP-21 on food intake, body weight, behavior, and oxygen consumption

In *ad libitum*-fed Siberian hamsters, ip administration of TLQP-21 (1, 5, and 25 mg/kg) at the onset of the dark phase did not affect intake of the palatable diet (Fig. 8, A–F) or body weight (Fig. 7G) at any time point investigated. Additionally, no sedation or hyperactivity or other adverse behaviors were observed in the first hour after ip administration of TLQP-21 (1, 5, or 25 mg/kg) when compared with saline-treated hamsters (Fig. 8H). We observed no significant changes in oxygen consumption (VO_2) after ip administration of TLQP-21 at any of the doses investigated (data not shown).

Discussion

Peptides encoded by the VGF gene were first implicated in the control of energy balance through targeted deletion of the VGF gene in mice (12). We have recently shown that VGF gene expression is photoperiodically regulated with a decrease in expression in the Arc but dramatically higher expression in the dorsomedial posterior Arc of Siberian hamsters in the lean state (24). To investigate the role of VGF in this species that displays seasonal regulation of energy balance, we have used a VGF-derived peptide, TLQP-21, as an experimental approach because recombinant VGF is not available and this fragment has been identified as one of the natural products of VGF processing in the rat brain (25). The principal findings of our studies are that single icv infusions of TLQP-21 decreased food intake and body weight throughout the 24-h period investigated and that chronic administration of this peptide produced a sustained and additive reduction in food intake such that by the final day of the study, food intake was reduced by 56% compared with hamsters receiving either the vehicle or a scrambled control peptide.

We have shown that the decrease in food intake induced by TLQP-21 is not a reflection of a decrease in the number or frequency of meals, nor does it result from induction of sedation or hyperactivity by the peptide. Rather, it is caused by a decrease in the food intake per feeding bout. We infer that the hamster's motivation to eat is not affected by the

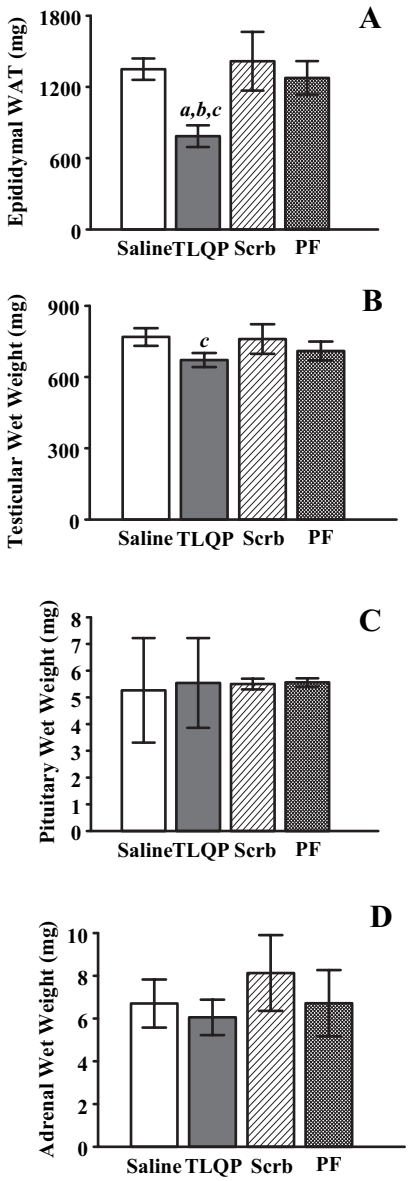

FIG. 6. Experiment 3: effects of chronic icv administration of TLQP-21 on wet tissue weight. Male Siberian hamsters fed *ad libitum* were treated daily before lights out (1100 h) with 1) 1 μl icv vehicle (saline), 2) 5 μg TLQP-21 in 1 μl (TLQP), or 3) 5 μg scrambled peptide in 1 μl (Scrb). In a fourth group, hamsters were treated daily with 1 μl icv saline, but their food intake was restricted to the median food intake of the TLQP-21-treated group, *i.e.* pair-fed (PF). After 7 d of treatment, wet weights of epididymal white adipose tissue (A), testes (B), pituitary glands (C), and adrenal glands (D) were obtained. Values are expressed as group means ± SEM (n = 8–11 per group), and significance values are indicated: *a*, $P < 0.001$ *vs.* saline; *b*, $P < 0.01$ *vs.* scrambled; *c*, $P < 0.05$ *vs.* saline.

peptide and that the effect of TLQP-21 results from a more rapid achievement of a state of satiation, reflected in a reduced food intake in each meal. The TLQP-21-induced decrease in food intake was accompanied by a sustained and progressive reduction in body weight compared with hamsters receiving saline or a peptide containing the same amino acids as TLQP-21 but in a scrambled sequence. The decrease in body weight appears to be mainly due to a decrease in white adipose tissue; after TLQP-21 treatment, we observed a 40% decrease in epididymal fat pad weight compared with control groups, a major intraabdominal fat depot in male hamsters (20).

To investigate the mechanism of action of TLQP-21, a separate group of hamsters received daily infusions of saline, but their food intake was restricted to that of the VGF-treated group. Body weight was reduced in these pair-fed hamsters compared with those treated with saline or scrambled peptide but was similar to that in the TLQP-21-treated group. This suggests that the principal effect of the TLQP-21 treatment on body weight was through an effect on food intake. In support, we have also shown there is no change in resting metabolic rate (measured as ml O_2/min·kg$^{0.75}$) after either single infusion or after multiple infusions of TLQP-21. We also investigated the effects of the VGF-derived peptide on gene expression in brown adipose tissue because this tissue is known to be a major contributor to the control of energy balance in rodents. In both TLQP-21-treated hamsters and in the pair-fed group, there was a substantial decrease in UCP-1 levels in brown adipose tissue that might be expected to result in reduced energy expenditure. Thus, activation of thermogenesis in brown adipose tissue in the TLQP-21-treated hamsters is not contributing to the catabolism of fat depots and loss of body weight. Indeed, the reduced capacity for thermogenesis after TLQP-21 treatment (and in the pair-fed group) more likely reflects a compensatory response induced by the reduced caloric intake. Although we did not measure circulating leptin levels in the current study, it is highly likely that they would have decreased in parallel with the decrease in abdominal fat pads as occurs naturally in hamsters in winter photoperiods (21–23). If so, the decrease in leptin may be the underlying cause of the decrease in UCP-1 mRNA, as is the case in mice (33). Reduced thermogenic capacity of hamsters after TLQP-21 treatment may indicate a contributory role of this peptide in the induction of torpor that hamsters undergo when a critical body weight loss has been achieved in winter photoperiods (34).

Our studies suggest that VGF exerts a net catabolic effect resulting from inhibition of food intake within the brain. This contrasts with the conclusion reached from studies in mice where VGF gene expression has been ablated and a lean hypermetabolic phenotype has been observed such that the loss of VGF is associated with a resistance to obesity and increased insulin sensitivity (11, 12, 35). Our findings are broadly consistent with the recent studies of Bartolomucci *et al.* (25) who observed slight increases in energy expenditure in mice receiving intracranial infusions of TLQP-21. Interestingly, no effects on food intake were observed in the study in mice when the animals were maintained on normal lab chow or high-fat diet (25). The Siberian hamsters appear to be more sensitive to the effects of TLQP-21 on food intake; in fact, we observed significant decreases in food intake even with a dose that was 3-fold lower than that used in mice. Small increases in metabolic rate were observed in mice on normal lab chow by Bartolomucci *et al.* (25), whereas we were unable to detect significant effects of the icv TLQP-21 treatment on oxygen consumption. The differences observed may be due to the two calorimetry systems measuring different parameters. In the majority of our studies, we used a closed-

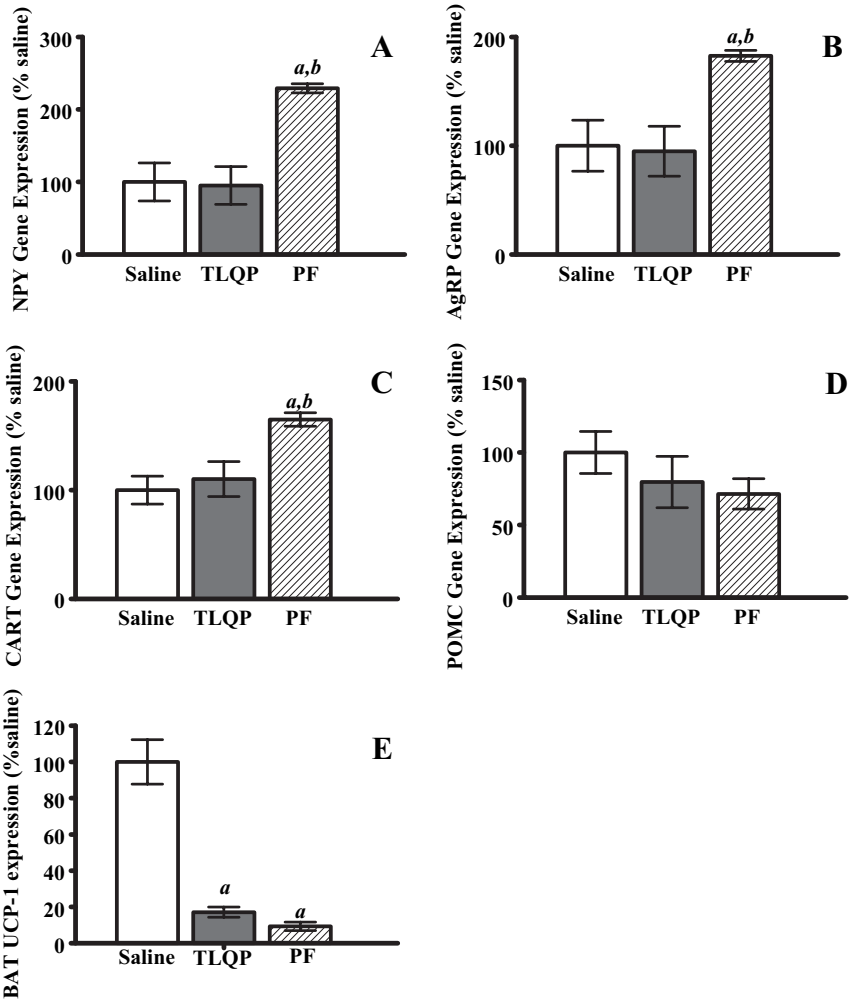

FIG. 7. Experiment 3: effects of chronic icv administration of TLQP-21 on hypothalamic gene expression. Male Siberian hamsters fed *ad libitum* were treated daily before lights out (1100 h) with either 1 μl icv vehicle (saline) or 5 μg TLQP-21 in 1 μl (TLQP). In a third group, hamsters were treated daily with 1 μl icv saline, but their food intake was restricted to the median food intake of the TLQP-21-treated group, *i.e.* pair-fed (PF). After 7 d of treatment, gene expression of NPY (A), AgRP (B), CART (C), and POMC (D) in the Arc and UCP-1 (E) in brown adipose tissue was determined. Values are expressed as means ± SEM (n = 8–11 per group), and significance values are indicated: *a*, $P < 0.001$ *vs.* saline; *b*, $P < 0.001$ *vs.* TLQP-21.

circuit system in which hamsters are maintained at thermoneutral temperature (29 C), which measures VO_2 (ml O_2/min·$kg^{0.75}$) when the animals are resting (or sleeping) and in a fasting state (there is no food or water available), providing a good measurement of resting metabolic rate. The studies in mice used the Columbus Instruments OxyMax system where the mice have *ad libitum* access to food and water, are maintained at room temperature, and have higher levels of locomotor activity (32); thus, overall metabolism is measured rather than specifically resting metabolic rate. However, we conducted one study in hamsters using the OxyMax system (experiment 2), and this also failed to detect a significant difference in VO_2 after TLQP-21 treatment, although the system was sensitive enough to detect significant changes in VO_2 across the light-dark cycle.

Despite the differences in actions of TLQP-21 between mice and hamsters, which could simply reflect species differences, the general tenor of the findings by both Bartolomucci *et al.* (25) and ourselves is that this VGF-derived peptide exerts an overall catabolic action, a substantially different conclusion from that reached by Hahm *et al.* (12) where studies in knockout mice suggested that VGF exerts anabolic effects. Bartolomucci *et al.* (25) consider the most likely explanation for the apparent contradiction between the functional *in vivo* studies of the effects of a VGF-derived peptide and studies where all VGF production has been ablated to be attributable to the fact that VGF polypeptide is known to be cleaved into multiple peptides, which could well have opposing biological effects. This is certainly the case for other recently discovered peptides; for example, ghrelin and obestatin are peptides encoded by the same gene but have opposite effects on food intake (36). We also postulated that VGF might exert pleiotropic effects in different tissues, because the VGF gene is expressed in many tissues in addition to the brain, including the pituitary, adrenal glands, gastrointestinal tract, and pancreas (37). We therefore investigated the role of systemic TLQP-21 on energy balance in the Siberian hamster by determining the effects of the VGF-derived peptide when administered peripherally. Despite the clear and robust effects of TLQP-21 when infused within the brain, we found that systemic administration of even large doses of TLQP-21 did not affect food intake and body weight or cause any hyperactivity or sedation or alter resting metabolic rate. Because no receptors for VGF-derived peptides have yet been described, we cannot speculate as to whether VGF gene expression in the periphery encodes products that are likely to act locally or signal to the brain, although on the basis of our current series of experiments, it

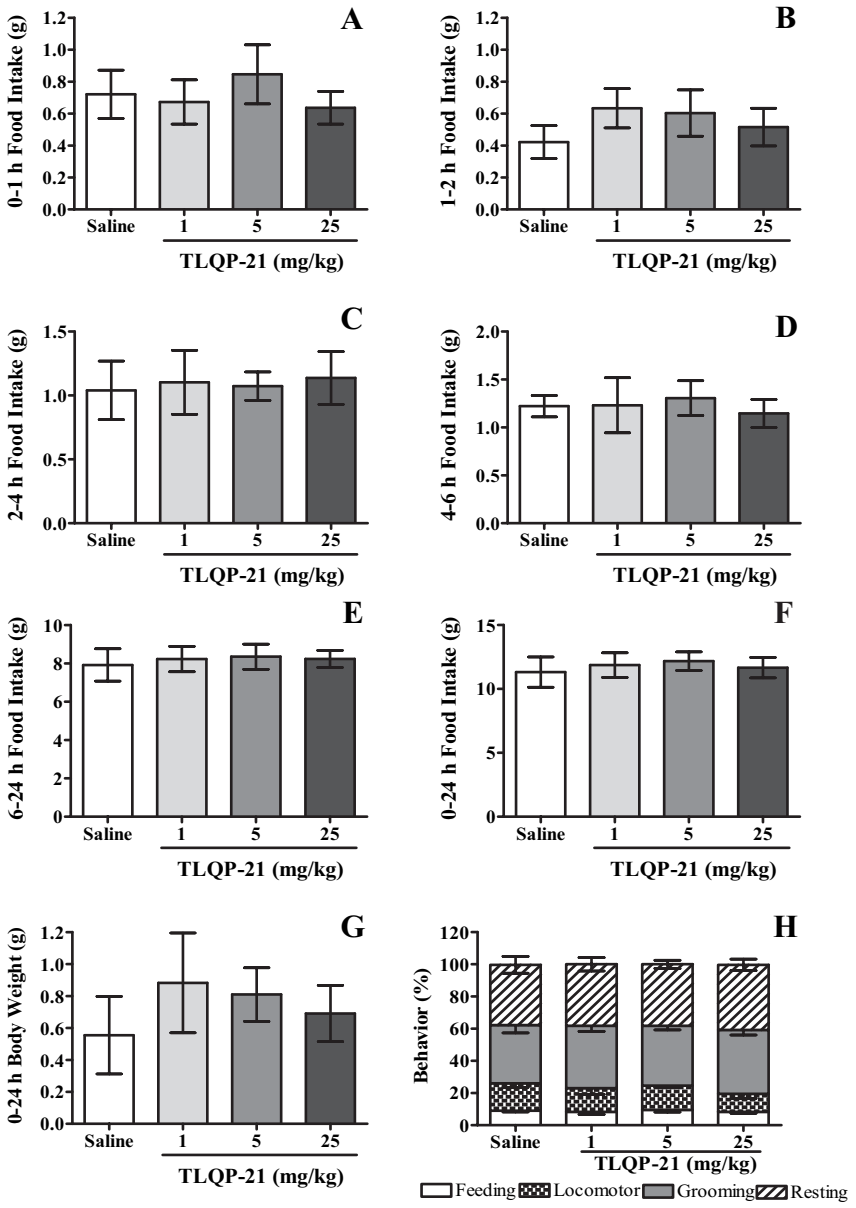

FIG. 8. Experiment 4: effects of acute systemic administration of TLQP-21 on food intake, body weight, and behavior. *Ad libitum*-fed male Siberian hamsters received an ip injection of either saline or VGF-derived peptide TLQP-21 (1, 5, or 25 mg/kg) over a 4-wk period so that each animal acted as its own control. Food intake was measured 0–1 h (A), 1–2 h (B), 2–4 h (C), 4–6 h (D), 6–24 h (E), and 0–24 h (F) after injection. Body weight (G) was measured before and 24 h after injection. Behavior (H) was measured 0–1 h after injection. *Bars* depict the proportion of observations of each of the predefined behaviors. All values are expressed as group means ± SEM (n = 11 per group).

seems highly unlikely that TLQP-21 crosses the blood-brain barrier. Whatever the sites of action, it is not surprising that loss of VGF in all tissues throughout the body (as in the VGF-knockout mice) might produce a net phenotype that masks the function of VGF-derived peptides at specific locations.

Because the primary action of TLQP-21 was to suppress food intake in hamsters, we investigated the effects of the chronic TLQP-21 infusion on the expression of genes in the Arc known to be implicated in the control of food intake. There was no significant effect of TLQP-21 treatment on two genes encoding orexigenic peptides (NPY and AgRP) or on POMC or CART. Some clear changes in gene expression were observed in the pair-fed controls, notably an up-regulation of NPY and AgRP mRNA, a compensatory response counteracting the effects of caloric restriction. There was no effect of food restriction (pair-fed group) on the expression of POMC, which is consistent with previous observations in Siberian hamsters (23). CART mRNA abundance was also increased in the Arc in the pair-fed group; this may seem unexpected because CART was originally thought to be an anorexigenic peptide (38), although studies in CART-knockout mice do not provide unequivocal evidence for this (39), and studies where CART infused directly into the hypothalamus of rats results in increases in food intake suggest that in certain localities, CART exerts an orexigenic effect (40). These dynamic changes in gene expression in the pair-fed hamsters demonstrate that our *in situ* hybridization technique is sufficiently quantitative to detect changes in states of altered energy balance, so it is of considerable interest that the TLQP-21 treatment, which clearly decreased food intake, did not affect expression of these feeding-related genes.

A very similar outcome was reported by Bartolomucci *et al.* (25) who used RT-PCR to assess mRNA abundance in

whole hypothalamic extracts from mice treated with TLQP-21 but found that expression of NPY, POMC, AgRP, MCH, and CRF were all unaffected, despite the catabolic phenotype of the treated mice. We infer that TLQP-21 does not act by altering expression of these genes in the Arc, so it is particularly interesting that it does affect hypothalamic function such that no compensatory response occurs. Thus, the pair-fed hamsters that were being food deprived displayed appropriate responses to counteracting the effects of caloric restriction, but the hamsters treated with TLQP-21, although eating less, were satiated in terms of the function of the Arc.

In summary, intraventricular infusion of the VGF-derived peptide TLQP-21 reduced food intake, resulting in a loss of intraabdominal white fat depots and decreased body weight and also a decrease in testicular weight. These physiological effects of TLQP-21 infusion are similar to that observed in Siberian hamsters during their natural transition from the summer obese state to the winter lean states. Because we have previously observed increased VGF mRNA abundance in the posterior Arc of Siberian hamsters in short photoperiods, we conclude that increased production of VGF-derived peptides in the hypothalamus may contribute to the development of the winter hypophagic and catabolic state observed in this species.

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References

1. **Levi A, Eldridge JD, Paterson BM** 1985 Molecular cloning of a gene sequence regulated by nerve growth factor. Science 229:393–395
2. **van den Pol AN, Bina K, Decavel C, Ghosh P** 1994 VGF expression in the brain. J Comp Neurol 347:455–469
3. **van den Pol AN, Decavel C, Levi A, Paterson B** 1989 Hypothalamic expression of a novel gene product, VGF: immunocytochemical analysis. J Neurosci 9:4122–4137
4. **Snyder SE, Salton SR** 1998 Expression of VGF mRNA in the adult rat central nervous system. J Comp Neurol 394:91–105
5. **Snyder SE, Pintar JE, Salton SR** 1998 Developmental expression of VGF mRNA in the prenatal and postnatal rat. J Comp Neurol 394:64–90
6. **Snyder SE, Li J, Salton SR** 1997 Comparison of VGF and trk mRNA distributions in the developing and adult rat nervous systems. Brain Res Mol Brain Res 49:307–311
7. **Possenti R, Eldridge JD, Paterson BM, Grasso A, Levi A** 1989 A protein induced by NGF in PC12 cells is stored in secretory vesicles and released through the regulated pathway. EMBO J 8:2217–2223
8. **Trani E, Ciotti T, Rinaldi AM, Canu N, Ferri GL, Levi A, Possenti R** 1995 Tissue-specific processing of the neuroendocrine protein VGF. J Neurochem 65:2441–2449
9. **Trani E, Giorgi A, Canu N, Amadoro G, Rinaldi AM, Halban PA, Ferri GL, Possenti R, Schinina ME, Levi A** 2002 Isolation and characterization of VGF peptides in rat brain. Role of PC1/3 and PC2 in the maturation of VGF precursor. J Neurochem 81:565–574
10. **Possenti R, Rinaldi AM, Ferri GL, Borboni P, Trani E, Levi A** 1999 Expression, processing, and secretion of the neuroendocrine VGF peptides by INS-1 cells. Endocrinology 140:3727–3735
11. **Hahm S, Fekete C, Mizuno TM, Windsor J, Yan H, Boozer CN, Lee C, Elmquist JK, Lechan RM, Mobbs CV, Salton SR** 2002 VGF is required for obesity induced by diet, gold thioglucose treatment, and agouti and is differentially regulated in pro-opiomelanocortin- and neuropeptide Y-containing arcuate neurons in response to fasting. J Neurosci 22:6929–6938
12. **Hahm S, Mizuno TM, Wu TJ, Wisor JP, Priest CA, Kozak CA, Boozer CN, Peng B, McEvoy RC, Good P, Kelley KA, Takahashi JS, Pintar JE, Roberts JL, Mobbs CV, Salton SR** 1999 Targeted deletion of the Vgf gene indicates that the encoded secretory peptide precursor plays a novel role in the regulation of energy balance. Neuron 23:537–548
13. **Schuhler S, Horan TL, Hastings MH, Mercer JG, Morgan PJ, Ebling FJ** 2004 Feeding and behavioural effects of central administration of the melanocortin 3/4-R antagonist SHU9119 in obese and lean Siberian hamsters. Behav Brain Res 152:177–185
14. **Schuhler S, Horan TL, Hastings MH, Mercer JG, Morgan PJ, Ebling FJ** 2003 Decrease of food intake by MC4-R agonist MTII in Siberian hamsters in long and short photoperiods. Am J Physiol Regul Integr Comp Physiol 284:R227–R232
15. **Schuhler S, Ebling FJ** 2006 Role of melanocortin in the long-term regulation of energy balance: lessons from a seasonal model. Peptides 27:301–309
16. **Mercer JG, Moar KM, Logie TJ, Findlay PA, Adam CL, Morgan PJ** 2001 Seasonally inappropriate body weight induced by food restriction: effect on hypothalamic gene expression in male Siberian hamsters. Endocrinology 142: 4173–4181
17. **Rousseau K, Atcha Z, Cagampang FR, Le RP, Stirland JA, Ivanov TR, Ebling FJ, Klingenspor M, Loudon AS** 2002 Photoperiodic regulation of leptin resistance in the seasonally breeding Siberian hamster (*Phodopus sungorus*). Endocrinology 143:3083–3095
18. **Reddy AB, Cronin AS, Ford H, Ebling FJ** 1999 Seasonal regulation of food intake and body weight in the male Siberian hamster: studies of hypothalamic orexin (hypocretin), neuropeptide Y (NPY) and pro-opiomelanocortin (POMC). Eur J Neurosci 11:3255–3264
19. **Ebling FJ, Cronin AS** 1998 Manipulations of glutamatergic (*N*-methyl-D-aspartate receptor) neurotransmission alter the rate of photoperiodically regulated sexual maturation in the male Siberian hamster. Biol Reprod 58:1–7
20. **Bartness TJ, Hamilton JM, Wade GN, Goldman BD** 1989 Regional differences in fat pad responses to short days in Siberian hamsters. Am J Physiol 257: R1533–R1540
21. **Klingenspor M, Dickopp A, Heldmaier G, Klaus S** 1996 Short photoperiod reduces leptin gene expression in white and brown adipose tissue of Djungarian hamsters. FEBS Lett 399:290–294
22. **Mercer JG, Moar KM, Ross AW, Hoggard N, Morgan PJ** 2000 Photoperiod regulates arcuate nucleus POMC, AGRP, and leptin receptor mRNA in Siberian hamster hypothalamus. Am J Physiol Regul Integr Comp Physiol 278: R271–R281
23. **Mercer JG, Moar KM, Ross AW, Morgan PJ** 2000 Regulation of leptin receptor, POMC and AGRP gene expression by photoperiod and food deprivation in the hypothalamic arcuate nucleus of the male Siberian hamster (*Phodopus sungorus*). Appetite 34:109–111
24. **Barrett P, Ross AW, Balik A, Littlewood PA, Mercer JG, Moar KM, Sallmen T, Kaslin J, Panula P, Schuhler S, Ebling FJ, Ubeaud C, Morgan PJ** 2005 Photoperiodic regulation of histamine H3 receptor and VGF messenger ribonucleic acid in the arcuate nucleus of the Siberian hamster. Endocrinology 146:1930–1939
25. **Bartolomucci A, La Corte G, Possenti R, Locatelli V, Rigamonti AE, Torsello A, Bresciani E, Bulgarelli I, Rizzi R, Pavone F, D'Amato FR, Severini C, Mignogna G, Giorgi A, Schinina ME, Elia G, Brancia C, Ferri GL, Conti R, Ciani B, Pascucci T, Dell'omo G, Muller EE, Levi A, Moles A** 2006 TLQP-21, a VGF-derived peptide, increases energy expenditure and prevents the early phase of diet-induced obesity. Proc Natl Acad Sci USA 103:14584–14589
26. **Ebling FJ** 1994 Photoperiodic differences during development in the dwarf hamsters *Phodopus sungorus* and *Phodopus campbelli*. Gen Comp Endocrinol 95:475–482
27. **Steward CA, Horan TL, Schuhler S, Bennett GW, Ebling FJ** 2003 Central administration of thyrotropin releasing hormone (TRH) and related peptides inhibits feeding behavior in the Siberian hamster. Neuroreport 14:687–691
28. **Schuhler S, Warner A, Finney N, Bennett GW, Ebling FJ, Brameld JM** 2007 TRH decreases feeding and increases body temperature, activity, and oxygen consumption in Siberian hamsters. J Neuroendocrinol 19:239–249
29. **Schuhler S, Clark A, Joseph W, Patel A, Lehnen K, Stratford E, Horan TL, Fone KC, Ebling FJ** 2005 Involvement of 5-HT receptors in the regulation of food intake in Siberian hamsters. J Neuroendocrinol 17:276–285
30. **Halford JC, Wanninayake SC, Blundell JE** 1998 Behavioral satiety sequence (BSS) for the diagnosis of drug action on food intake. Pharmacol Biochem Behav 61:159–168
31. **Jones HN, Ashworth CJ, Page KR, McArdle HJ** 2006 Cortisol stimulates system A amino acid transport and SNAT2 expression in a human placental cell line (BeWo). Am J Physiol Endocrinol Metab 291:E596–E603
32. **Adam CL, Moar KM, Logie TJ, Ross AW, Barrett P, Morgan PJ, Mercer JG**

2000 Photoperiod regulates growth, puberty and hypothalamic neuropeptide and receptor gene expression in female Siberian hamsters. Endocrinology 141:4349–4356
33. **Commins SP, Watson PM, Padgett MA, Dudley A, Argyropoulos G, Gettys TW** 1999 Induction of uncoupling protein expression in brown and white adipose tissue by leptin. Endocrinology 140:292–300
34. **Bartness TJ, Elliott JA, Goldman BD** 1989 Control of torpor and body weight patterns by a seasonal timer in Siberian hamsters. Am J Physiol 257:R142–R149
35. **Watson E, Hahm S, Mizuno TM, Windsor J, Montgomery C, Scherer PE, Mobbs CV, Salton SR** 2005 VGF ablation blocks the development of hyperinsulinemia and hyperglycemia in several mouse models of obesity. Endocrinology 146:5151–5163
36. **Zhang JV, Ren PG, vsian-Kretchmer O, Luo CW, Rauch R, Klein C, Hsueh AJ** 2005 Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. Science 310:996–999
37. **Salton SR, Ferri GL, Hahm S, Snyder SE, Wilson AJ, Possenti R, Levi A** 2000 VGF: a novel role for this neuronal and neuroendocrine polypeptide in the regulation of energy balance. Front Neuroendocrinol 21:199–219
38. **Kristensen P, Judge ME, Thim L, Ribel U, Christjansen KN, Wulff BS, Clausen JT, Jensen PB, Madsen OD, Vrang N, Larsen PJ, Hastrup S** 1998 Hypothalamic CART is a new anorectic peptide regulated by leptin. Nature 393:72–76
39. **Moffett M, Stanek L, Harley J, Rogge G, Asnicar M, Hsiung H, Kuhar M** 2006 Studies of cocaine- and amphetamine-regulated transcript (CART) knockout mice. Peptides 27:2037–2045
40. **Abbott CR, Rossi M, Wren AM, Murphy KG, Kennedy AR, Stanley SA, Zollner AN, Morgan DG, Morgan I, Ghatei MA, Small CJ, Bloom SR** 2001 Evidence of an orexigenic role for cocaine- and amphetamine-regulated transcript after administration into discrete hypothalamic nuclei. Endocrinology 142:3457–3463

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