Genes Nutr (2007) 2:169–180
DOI 10.1007/s12263-007-0047-0

REVIEW

The role of the *vgf* gene and VGF-derived peptides in nutrition and metabolism

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Received: 15 September 2006 / Accepted: 24 October 2006 / Published online: 18 October 2007

Abstract Energy homeostasis is a complex physiological function coordinated at multiple levels. The issue of genetic regulation of nutrition and metabolism is attracting increasing interest and new energy homeostasis-regulatory genes are continuously identified. Among these genes, *vgf* is gaining increasing interest following two observations: (1) VGF-/- mice have a lean and hypermetabolic phenotype; (2) the first VGF-derived peptide involved in energy homeostasis, named TLQP-21, has been identified. The aim of this review will be to discuss the role of the *vgf* gene and VGF derived peptides in metabolic and nutritional functions. In particular we will: (1) provide a brief overview on the central systems regulating energy homeostasis and nutrition particularly focusing on the melanocortin system; (2) introduce the structure and molecular characteristic of *vgf*; (3) describe the phenotype of VGF deficient mice; (4) present recent data on the metabolic role of VGF-derived peptides, particularly focusing on one peptide named TLQP-21.

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Introduction

Energy homeostasis is a complex physiological function coordinated at multiple levels [5, 45, 47]. Stimulated by the discovery of leptin and the pandemic diffusion of obesity and type 2 diabetes, the regulation of energy homeostasis now receives increasing interest [26, 28, 29]. Novel genes are continuously identified with a role in energy homeostasis [18, 56, 59, 68, 96]. This leads to a growing need for new tools to improve the completeness of nutrigenomic studies [42]. The aim of this review will be to focus on one such genes, namely *vgf*. In particular we will: (1) provide a brief overview on the central systems regulating energy homeostasis and nutrition particularly focusing on the melanocortin system; (2) introduce the structure and molecular characteristic of *vgf*; (3) describe the phenotype of VGF deficient mice; (4) present recent data on the metabolic role of VGF-derived peptides, particularly focusing on one peptide named TLQP-21.

Hypothalamic and autonomic control of nutrition and metabolism

The hypothalamus is the core of the central circuits predisposed to regulate energy homeostasis and nutrition by sensing the level and the activity of central and peripheral mediators and activating catabolic/anabolic pathways [36, 53, 77]. The arcuate nucleus (ARC) of the hypothalamus is located in the center of this system: (1) it express

leptin/insulin/ghrelin (among the others) receptors; (2) activates catabolic/anabolic pathways mediating increased/decreased energy expenditure and altering food intake trough distinct downstream neural pathways [22]. Activation of different sub-population of ARC nuclei determine opposite effects. Activation of proopiomelanocortin (POMC) or neuropeptide (NP)Y/agouti-related-peptide (AGRP) cells determines the activation of catabolic pathways leading to fasting/energy expenditure or anabolic pathways leading to feeding/energy conservation, respectively [22, 53, 77]. Anabolic signals downstream of NPY/AGRP ARC cells target other hypothalamic nuclei including the lateral hypothalamus (LH) and the perifornical area. Here peptide signaling includes MCH, orexin and hypocretins. These neuropeptides stimulate to a different extent food intake, intestinal motility and stimulate behavioral activities involved in energy conservation [22, 53, 77].

On the contrary, fasting/energy dissipating pathways downstream of the ARC encompass the hypothalamic periventricular (PVN) and LH nuclei, which than projects to other brain area or the pituitary and leads to the coordinated activation of: (1) the sympathetic innervations to metabolic tissues (2) the release of epinephrine from the adrenal medulla; (3) the thyroid axis; (4) substrate oxidation in different tissues; (5) behavioural energy-dissipating activities; (6) inhibition of feeding. Synergistic activation of these pathways finally leads to increased energy expenditure and dissipation (rise in body temperature), as well as lipolysis [36, 47, 77].

Peripheral target tissues of these pathways include metabolic tissues such as muscles, liver, as well as the brown (BAT) and the white adipose tissue (WAT) which were the focus of several excellent reviews to which readers are remanded for further details [16, 20, 58, 87].

The melanocortin system

The central melanocortin system is perhaps the best-characterized central pathway regulating energy metabolism [22, 26]. This collection of circuits is unique in having the capability of sensing signals from many hormones, nutrients and afferent neural inputs. In the mammalian brain the melanocortin system is defined by: (1) neurons that express hypothalamic NPY and AGRP that originate in the ARC; (2) neurons within the ARC that express POMC, from which the peptide α-melanocyte-stimulating-hormone (α-MSH) is cleaved; (3) brainstem POMC neurons originating in the commissural nucleus of the solitary tract (NTS) and (4) downstream targets of these POMC and AGRP neurons expressing the melanocortin-3 (MC3R) and MC4R. In the CNS, α-MSH is agonists of the MC3R and MC4R, whereas AGRP is a high-affinity antagonist of both these receptors. The melanocortin system is regulated by complex cross-inhibitory signals in that MCR-expressing cells receive projections from both POMC and AGRP containing fibers.

It has been reported that reduced activity of the CNS melanocortin pathway promotes fat deposition via both food intake-dependent and -independent mechanisms [1]. Interestingly very recent evidences obtained in MC4R-/- mice suggest a divergent melanocortin pathway in the control of food intake (hypothalamic/amigdaloid nuclei) and energy expenditure (unknown site, likely brainstem or spinal cord nuclei) [7]. This relevant observation led Balthasar et al. [7] to conclude that central regulation of energy balance can be now viewed as having three elements: (1) a sensory, afferent arm receiving inputs from the gut, adipose tissue, and metabolic factors, (2) an integrative component where this sensory information is processed along with inputs from higher centers in the brain, and (3) an efferent arm that splits at some level to control food intake and energy expenditure. Divergence of melanocortin signaling, with respect to regulation of food intake and energy expenditure, places MC4Rs on the efferent side of this system [7].

The *vgf* gene and VGF-derived peptides

The number of genes involved in the regulation of nutrition and metabolism is enormous [69]. Among these genes, *vgf* is gaining increasing interest following two observations: (1) VGF-/- mice have a lean and hypermetabolic phenotype [33, 34], (2) the first VGF-derived peptide involved in energy homeostasis has been identified [11].

Vgf has been initially identified as a nerve growth factor (NGF)-inducible transcript in PC12 cells [48]. VGF has a tissue-specific pattern of expression limited to neurons within the central and peripheral nervous system and to various endocrine cells [49, 75]. In the adult rat brain, VGF mRNA is particularly abundant in the olfactory system, cerebral cortex, hypothalamus and hippocampus, and in a number of thalamic, septal, amygdaloid and brainstem nuclei. Following colchicines treatment to block anterograde transport of vesicles to nerve terminals, VGF-immunoreactivity is present in neurosecretory regions of the hypothalamus, including the ARC, PVN and supraoptic and suprachiasmatic nuclei [88]. In the PNS, VGF is highly expressed in both neurons of sympathetic ganglia and primary sensory neurons. VGF mRNA has been detected in the adult rat spinal cord, in α and γ motoneurons of the ventral horn, and in the dorsal horn neurons, as well as in cells of the inner nuclear and ganglion cell layers of the retina [25, 78]. Endocrine cell types that express VGF include posterior pituitary, as well as neuroendocrine and

endocrine cells of the anterior pituitary, adrenal medulla, gastrointestinal tract and β-cells pancreatic islets [25, 75]. Consistent with the reported VGF distribution, expression of VGF in cultured cell lines is limited to those derived from neuronal and neuroendocrine tissues [72].

The *vgf* gene encodes a precursor protein of 615 (human) and 617 (rat, mice) amino acids [49, 75]. VGF precursor protein sequence is highly conserved among rats and mice, with only 21 out of 617 amino-acids substitutions, none of which occur at the C-terminus. Most importantly for the results that will be discussed the in the last section of the review, the aminoacid sequence of the TLQP-21 peptide is identical in rat and mice and also highly conserved in humans.

Early studies in PC12 cells demonstrated that VGF is routed to the secretory compartment and released in response to depolarizing stimuli [64]. A major feature of VGF is the presence of specific sequence with basic amino acid residues that represent potential cleavage sites for proprotein convertases of the kexin/subtilisin-like serine proteinase family. Of note, the positions within the VGF polypeptide of each of these ten pairs of basic residues are highly conserved across species so far investigated. Upon processing by the neuroendocrine-specific prohormone convertases PC1/3 and PC2, VGF may yield a number of peptides that are stored in dense core granules and secreted through the regulated pathway [86]. In PC12 cells VGF is detected by Western blot analysis as a doublet of 80–90 kDa. Besides the 80–90 kDa doublet, antibodies raised against the C-terminal nonapeptide of rat VGF protein identify a number of smaller peptides in brain homogenates, and in extracts of primary cultures of cerebellar granule cells and of neuronal, endocrine, and pancreatic cell lines [65, 85]. By convention the VGF derived peptides are designated by the four N-terminal amino acids and the total length [49]. The most prominent VGF-derived peptides have apparent molecular masses of 20 and 10 kDa (named NAPP129 and TLQP-62, respectively), while others of 18 and 6 kDa (HHPD51) are also often detected. Other tissues (e.g., adrenal medulla) and cell lines (e.g., pituitary-derived GH3 and neural crest-derived PC12) produce substantial amounts of the 80–90 kDa doublet in the absence of measurable levels of the low-molecular-weight species. Small VGF peptides were shown to be generated by endoproteolytic cleavage in a late compartment of the secretory pathway [85]. The first VGF peptide identified was AQEE-30, also known as Peptide-V identified in bovine posterior pituitary [51]. Presently known C-terminal VGF-derived peptides are summarized in Fig. 1. In addition, several N-terminal fragments were found in human cerebrospinal fluid [62, 80, 95]. Finally, it is worth noting that a recent investigation also showed that processing of the neuropeptide precursor VGF was also affected in dwarf PC1 knockout mouse brains with a decrease in the level of an endogenous 3 kDa C-terminal peptide [61].

Quantification of VGF-derived peptides within brain structures is still in its infancy. Two recent studies provided data showing a differential amount of AQEE-30 in different brain regions and allowed to extrapolate an amount of at least 20 pmol/brain for this peptide [19, 30]. However the radioimmunoassay kit is not selective for AQEE-30 because also detects with an affinity of 90% TLQP-62 and the precursor protein [19].

Up to now, four VGF peptides were shown to possess biological activity: TLQP-62 and AQEE-30 increase the synaptic charge in hippocampal neurons [2]; AQEE-30 and LQEQ-19 facilitate penile erection in rats [82, 83]; TLQP-21 induces contractile responses in isolated gastric longitudinal muscle by stimulating the production of prostanoids (Severini et al., unpublished), modulates formalin pain (Rizzi et al., unpublished) and, as will be described below, regulate energy homeostasis [11]. In addition, in a recent report obese carboxypeptidase ($Cpe^{fat/fat}$) mice showed a reduced level of four fragments of VGF in the prefrontal cortex [50]. Therefore VGF can be viewed as a polypeptide precursor encoding for different physiologically active neuropeptides in analogy with others, e.g., POMC.

The *vgf* gene modulates nutrition and metabolism: evidences from VGF knockout mice

As mentioned ARC NPY and α-MSH expressing cells primarily control the activation of anabolic/catabolic pathways. The first evidence linking *vgf* with nutrition was that mice in the fed state showed colocalization of VGF mRNA with α-MSH in ARC nuclei. During fasting, VGF expression generally increases in the ARC where its co-localization increased with NPY and decreases with POMC [33, 34]. In addition, VGF mRNA levels are induced in response to light stimulation in the SCN the site of the mammalian circadian pacemaker also involved in metabolic regulation [81, 92]. More recently changes in VGF expression was found to differ in ARC nuclei of siberian hamsters exposed to short or long day's length preceding metabolic and body weight changes [8, 71].

Beside these studies, much of the information for a role of *vgf* in nutrition and energy homeostasis derive from reports on VGF-/- mice developed in 1999 by Salton et al. [33]. VGF deficient mice were indistinguishable from wild types at birth, but showed a reduced growing curve resulting in smaller dimension than wild type littermates: by postnatal day 3 (PND3), VGF mutant pups weighed 10–20% less; by PND21, 40–60% less than wild types; after weaning, VGF mutant mice maintained body weights

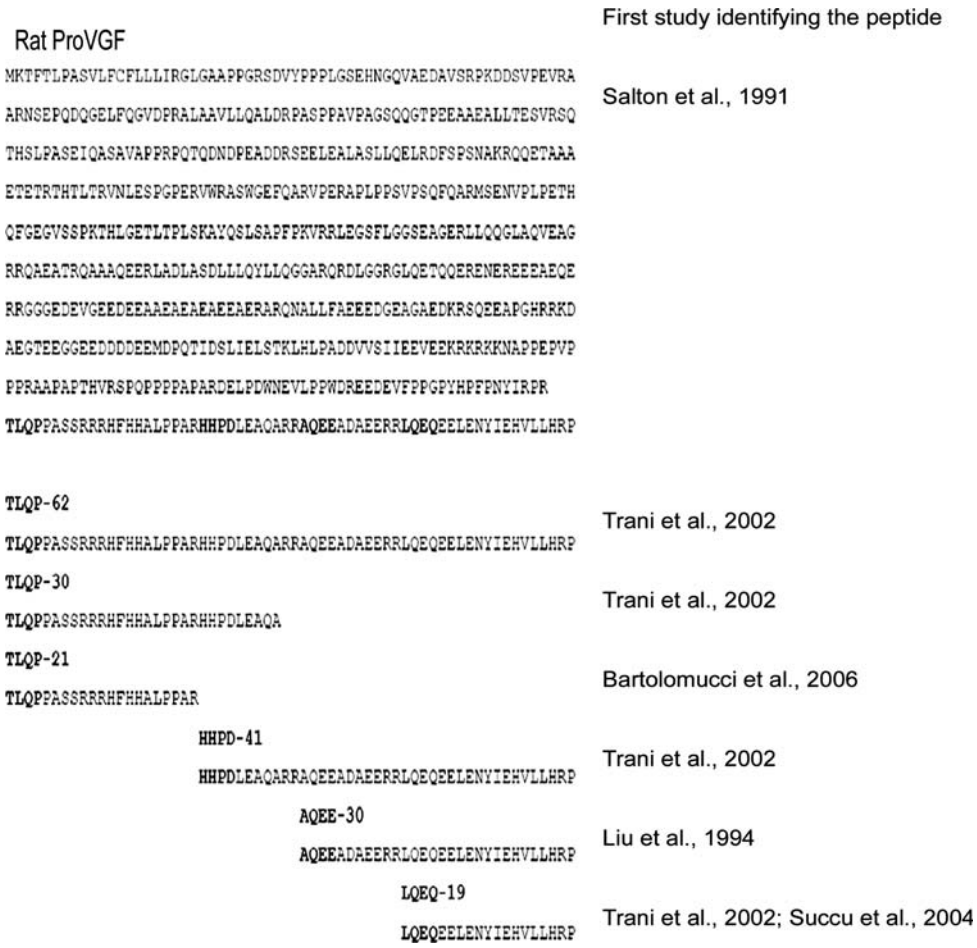

Fig. 1 The aminoacid sequence of the proVGF polypeptide and C-terminal VGF-derived peptides along with the first study where the specific peptide was first described

50–70% those of wild type. In addition to being smaller, VGF deficient mice were hypermetabolic by showing approximately 50% more O_2 consumption than wild types. Importantly, daily food intake was similar to those of wild types when expressed in absolute grams of ingested food, while this resulted in an elevation in food intake per gram of body weight in VGF-deficient compared with wild type mice. On the contrary, comparable hyperphagic responses were noted following a 24 h fast.

These changes in metabolic and nutritional parameters are paralleled by a peculiar endocrine and hematological profile [33, 34, 90]. Indeed, in ad lib fed VGF mutant mice mean serum insulin and glucose levels were 20 and 40% lower than normal mice, while the mean serum corticosterone level of VGF mutant mice was 40% increased, all consistent with a fasted state. VGF-deficient mice are also more insulin sensitive by showing a greater and more prolonged decrease in their plasma glucose levels following insulin injection [90].

Metabolic and nutritional changes were paralleled by molecular changes of hypothalamic neuropeptides. In VGF-/- mice, hypothalamic levels of NPY and AGRP mRNAs were elevated by 600 and 800%, respectively, while POMC mRNA was reduced by 75% in comparison with controls which is compatible with a fasting state [77].

These findings prove the *vgf* gene to be a key regulator of energy homeostasis and nutrition in both basal and fasting/re-fed protocols. A further confirmation of the key role played by VGF comes from experimental models of obesity. Indeed it was proved that VGF is required for obesity induced by diet, gold thioglucose (GTG) treatment and agouti ectopic overexpression [34]. In fact, ablation of the *vgf* gene blocked the metabolic effects of the high-fat diet on body and fat-pads weight, as well as changes in adipose leptin mRNA or circulating leptin. In addition, mean plasma glucose levels were higher in both mutant and wild type mice fed the high calorie diet, while circulating insulin and leptin levels were elevated in wild type mice but unchanged in VGF mutant mice fed high calories [90]. Another widely used model of obesity is that caused by GTG [14]. Targeted deletion of the *vgf* gene completely prevented the increase in body weight, hyperphagia, obesity and hyperglicemia produced by GTG treatment in normal mice [34, 90].

A classical genetic form of obesity is due to ablation of the leptin gene or its receptor [73]. Targeted deletion of *vgf*

completely blocked the effects of leptin deficiency on hyperphagia, food intake and glucose level and attenuated body weight gain in VGF-/- Ob/ob double-mutant mice. *ob/ob*, *Vgf*-/- mice had insulin levels intermediate between ob/ob and wild type mice [90]. However, ablation of the *vgf* gene did not prevent the development of increased adiposity or reduced body temperature in ob/ob mice. Agouti-mediated obesity results from ectopic overexpression of the agouti polypeptide, a melanocortin receptor blocker that decreases normal satiety signaling by α-MSH [9]. Ablation of the *vgf* gene in A*y*/*a* mice completely suppressed the obese phenotype. Plasma glucose levels were significantly decreased in VGF-deficient mice with or without the agouti mutation in comparison to wild type or agouti mutant mice while serum insulin levels in double mutant mice were significantly lower than those measured in *Ay*/*a* agouti mice and significantly higher than VGF mutant mice, but were not significantly different from control levels [90].

These differences in double mutant *Ay*/*a*, Vgf-/Vgf- and *Ay*/*a* mice suggested that VGF might function in pathways downstream to the MC4-R that project via the autonomic nervous system to peripheral metabolic tissues. Further support from this observation came from the last experimental model of obesity used by Hahm et al. namely repetitive daily injections of MSG administered to neonatal mice from PND2 to PND12 [63]. In this model obesity results from damage to the hypothalamus, the sympathetic nervous system including the innervation of BAT that could disrupt thermogenesis and lead to increased adiposity [14, 55]. Interestingly, in contrast to all the other forms of obesity examined targeted deletion of the *vgf* gene had little influence on the ability of MSG treatment to increase body weight but determined a clear hyperglicemia [34, 90]. These results therefore strengthen the findings with double mutant *Ay*/*vgf* mice and further support a role for VGF downstream of hypothalamic/autonomic centers.

VGF-derived peptides modulate nutrition and metabolism

The phenotype of VGF-/- mice while clearly suggesting a metabolic role for *vgf* does not clarify which are the molecular mediators of its effect among the several VGF-derived peptides. We recently started a project to clarify the biological role of VGF-derived peptides. The bulk of evidences available so-far regards the C-terminal VGF internal peptide TLQP-21 which has been recently identified in the rat brain by of immunoprecipitation, microcapillary liquid chromatography–tandem mass spectrometry and database searching algorithms [11]. We have previously shown that TLQP-62 is efficiently produced into dense core granules upon processing by the prohormone convertase PC1/3 at a site that does not conform to the classical PC dibasic target motif [86]. Similarly, it is conceivable that TLQP-21 could be produced by the action of prohormone convertases at the PPARHH even though it does not matches a consensus prohormone convertase target site. An intriguing alternative possibility is that TLQP-21 is generated by extracellular proteases acting on secreted VGF forms [44].

Following its identification, TLQP-21 was chronically delivered icv for 14 days with osmotic minipumps and nutritional and metabolic effects investigated in mice in two conditions, i.e., with standard rodent chow and in high fat diet. In mice fed a standard diet TLQP-21 treatment influenced energy expenditure, adrenergic function, and lipid profile, while body weight and food intake were unaffected (Fig. 2). In particular we proved that TLQP-21 increased energy expenditure and rectal temperature, an effect which was paralleled by increased serum epinephrine or decreased norepinephrine level, being instead, independent from locomotor activity, fT3 and fT4 serum level. Hematological biomarkers showed a consistent profile. TLQP-21 treatment lowered triglycerides (TG) while free fatty acids (FFA) and glucose level remained unaffected, therefore an increased FFA/TG ratio was observed. These changes in TLQP-21 treated mice occurred despite a non-significant reduction of WAT/bw and circulating leptin. Therefore, central TLQP-21 may upset energy balance in mice. Which would be the primary mechanism trough which TLQP-21 exert its action? A clear answer to this question still remains elusive because it is yet unknown: (1) which brain nuclei produce and release TLQP-21; (2) which is the receptor of TLQP-21. However, based on current knowledge, some hypotheses can be formulated. Severini et al. (unpublished) showed that TLQP-21 stimulated gastric fundus strips contraction, an effect which was inhibited by pre-treatment with the non-selective cyclooxygenase (COX) inhibitors indomethacine and naproxen and by the PGF2dimethyl-amide and SC-19220 which act as FP and EP1 prostaglandin receptor antagonists, respectively. In addition, production of prostaglandin E2 (PGE2), PGF2α and PGD2 was detected in the medium following co-incubation of rat fundus dissections with TLQP-21. Central prostaglandins (PGE2 in particular) induction or treatment is known to induce hyperthermia and to increase energy expenditure [3, 35, 43]. Therefore chronic TLQP-21-induced upset of energy balance is compatible with a mechanism of action involving CNS COX-2 stimulation and PG production. Interestingly these changes appear to be independent from changes occurring in major hypothalamic anorexigenic and orexigenic neuropeptide AGRP, NPY, MCH, POMC and CRH [11]. Therefore, our results would rule out a primary role of the

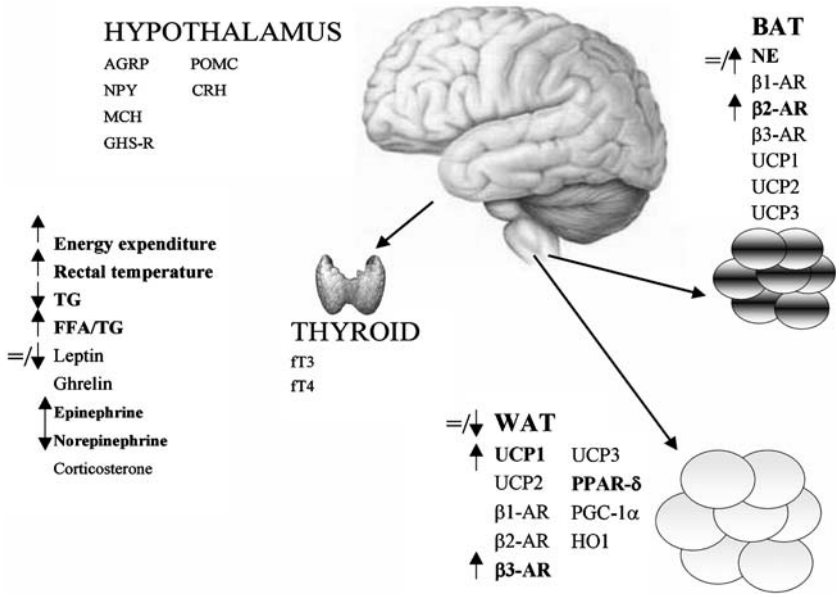

Fig. 2 Diagrammatic drawing of the effects induced by chronic central TLQP-21 treatment. ↑, ↓ and =/↑ and *bolded fonts* represent significantly increased, decreased and slightly increased parameters, respectively

hypothalamus in mediating the effects of TLQP-21 while being compatible with the activation of brainstem nuclei downstream of the hypothalamus. This conclusion agrees with the proposal that VGF would function in the outflow pathways downstream of hypothalamic melanocortin 4 receptors (MC4R) that project via the autonomic nervous system (ANS) to peripheral metabolic tissues [34, 49]. As mentioned, Balthasar et al. [7] described a divergent melanocortin pathway in the control of food intake and energy expenditure. In particular, the brain regions responsible for melanocortin-induced increase of energy expenditure might be brainstem and spinal cord neurons in which MC4R colocalizes with pseudorabies virus injected in the inguinal white adipose tissue of sirian hamsters [79]. TLQP-21, not affecting either feeding or hypothalamic peptide mRNAs, but affecting energy expenditure, may function in one such MC4R regulated extra-hypothalamic sites regulating energy expenditure. In agreement with this hypothesis we showed increased catabolic markers in the BAT and WAT following TLQP-21. In detail, changes in the BAT were limited to increased β2-AR expression. On the contrary, molecular analysis of the WAT demonstrated substantial molecular changes: PPAR-δ,(β3-AR, and the brown adipocytes specific UCP1 mRNA were up-regulated. WAT receives sympathetic innervation downstream of PVN and VMH hypothalamic nuclei, the nucleus of the solitary tract, the intermediolateral cell group and the central autonomic nucleus of the spinal cord [10, 15, 27, 66]. Sympathetic stimulation determines lipolysis and energy expenditure primarily via β-adrenergic stimulation [4, 41, 46]. In agreement with this proposal, following TLQP-21 treatment WAT weight slightly decreased while β3-AR gene expression was up-regulated within the same tissue, with the two parameters being inversely correlated, and being inversely correlated with norepinephrine tissue content. TLQP-21 treatment, in addition to β3-AR, also resulted in increased PPAR-δ gene expression in the WAT, which may also contribute to the observed increase in energy expenditure. Supporting this it has been reported that PPAR-δ determines fatty acid oxidation and energy uncoupling in WAT [16, 24, 89]. Finally TLQP-21 also increased UCP1 mRNA in the WAT. Fat pads in mammals are a mix of brown (expressing UCP1) and white (not expressing UCP1) adipocytes with a site-specific prevalence of one or the other [20]. The epididymal (or perigonadal) WAT is mainly constituted of white adipocytes. Therefore, increased UCP1 gene expression after TLQP-21 treatment would imply transdifferentiation of brown adipocytes in the WAT [29, 60, 84].

Therefore, central TLQP-21 infusion upsets energy balance but did not determine an overall shift in energy homeostasis, as proved by the lack of changes in body weight. Important, Jethwa et al. [39], in an abstract presented at the meeting of Neuroendocrinology, showed that TLQP-21 could affect energy homeostasis in siberian hamster. Therefore, these data support and extend our observation by demonstrating that TLQP-21 has catabolic effects in rodents.

Following our observations in mice we hypothesized that TLQP-21 could affect energy balance when energy homeostasis is boosted by a hypercaloric diet consisting of 14 days high fat diet (20% lard addition; HF). Diet-induced obesity starts to develop in control animals (HF-CON), which at the end of the experiment showed increased body weight gain, increased caloric efficiency and hypertrophy of visceral WAT when compared with mice receiving a

standard diet. Without any difference in Kcal ingested by mice eating high fat diet and treated with TLQP-21 (HF-TLQP-21) or vehicle (HF-CON), TLQP-21 treatment prevented development of diet-induced obesity (Fig. 3). Indeed, HF-TLQP-21 treated mice only showed a modest and non-significant increase in body and WAT weight, and caloric efficiency when compared to ST-CON. Endocrine biomarkers were consistent with the obesity-like phenotype of HF-CON mice: serum leptin increased, while ghrelin decreased. HF-TLQP-21 mice instead showed approximately half, and non-significant, rise of leptin showed by HF-CON mice and normalization of ghrelin. TLQP-21-induced increase of EE, T and adipose tissue catabolic mediators, is compatible with the block of weight gain and adiposity [52, 53, 77].

It is worth noting that HF-CON mice showed changes in pattern of hypothalamic gene expression consistent with the development of obesity, i.e., up-regulated MCH and POMC (Bartolomucci et al., unpublished). These effects have been previously described in the early phase of diet induced obesity [23, 98] and are understood in terms of compensatory increase in energy expenditure (e.g., POMC) or enhanced appetite for a palatable diet (e.g., MCH). HF-TLQP-21 mice showed a complete normalization of hypothalamic mRNA changes observed in HF-CON mice while showing a decrease in growth-hormone-secretagogues-receptor (GHS-R) expression (Bartolomucci et al., unpublished), the receptor for GH segretagogues, which has been identified as a hypothalamic regulator of anabolic functions [57, 97]. These effects at the hypothalamic level is similar to those discussed above for mice fed a standard diet, which would rule out a primary involvement of hypothalamic peptides in the action of TLPQ-21, the only exception being the inhibition of GHS-R expression.

In conclusion, our study identified for the first time a metabolic role for a recently identified VGF-derived peptide, TLQP-21. Overall, results discussed address a role for this peptide in centrally stimulating the autonomic nervous system, possibly via central PG induction and peripheral adrenomedullary activity and adipose tissue catabolism, to upset energy balance. By virtue of its effect, TLQP-21 also limited weight gain and adiposity associated with high-fat diet.

Surprisingly, the profile of TLQP-21 treated mice closely matches the phenotype of the VGF-/- mice [11, 33, 34]. It is not unusual that results from constitutive gene-knockout and pharmacological studies determine contrasting findings, and this is particularly true when knockout mice are produced before a given function is addressed for the gene products, as is the case for *vgf* [13, 31, 32, 76]. Well-known examples of contradictory findings concern 5HT-1B and POMC. 5HT-1B knockout mice exhibit an increased, while 5HT-1B agonists treated subjects exhibit a decreased locomotory response to cocaine [17, 70].

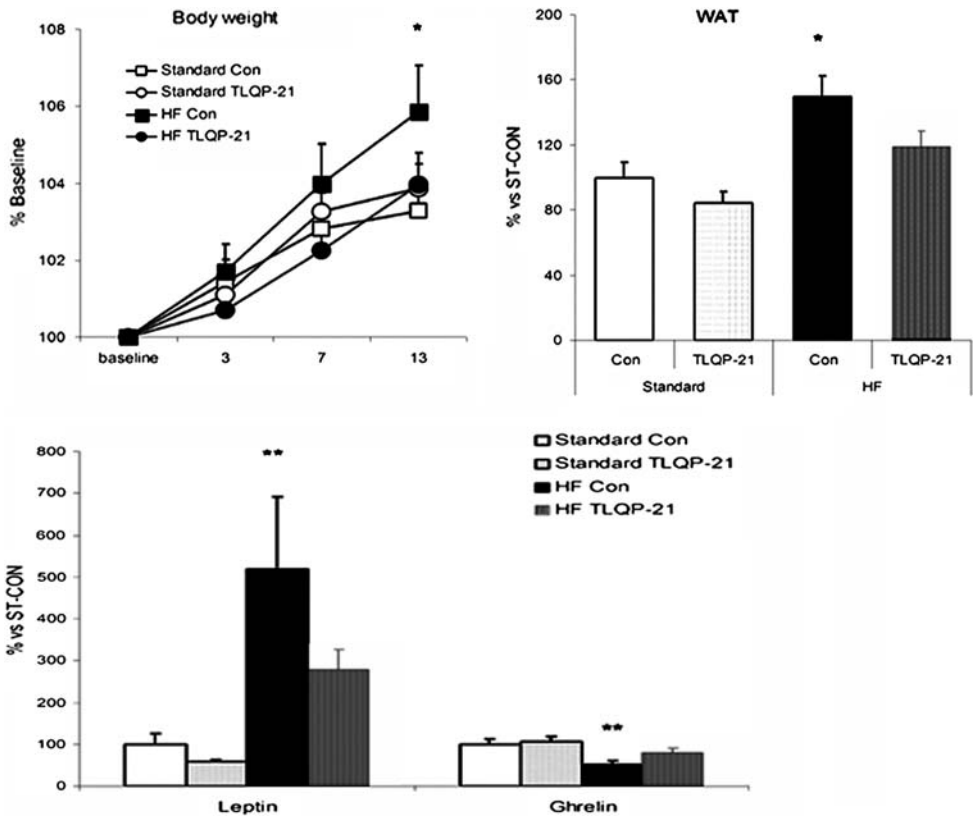

Fig. 3 Physiological effects of chronic central TLQP-21 in mice fed high-fat diet. *Upper left* changes in body weight; *Upper right* changes in white adipose tissue weight; *Lower* changes in circulating leptin and ghrelin. Adapted from [11]

POMC-deficient mice are obese and hyperphagic and do respond to melanocortin agonists treatment, while β-endorphin treatment results in hyperphagia [6, 94]. In both cases the advocated explanation to the apparent paradox regards the alteration in other brain systems than the targeted one.

However, an intriguing hypothesis would resolve the contradictory finding of our and Hahm et al. studies: one or more VGF-derived peptides should have an anabolic role positively affecting energy homeostasis. Recently we started to investigate this hypothesis by focusing on other C-terminal VGF-derived peptides than TLQP-21, i.e., TLQP-62 and HHPD-41 spanning from residues 556–617 and 577–617 of ProVGF sequence, respectively. TLQP-62, also known as VGF-10, represent the 62-aminoacid carbossi terminus of VGF which was identified by Trani et al. [86] in cultured PC12 cells and thereafter shown to modulate the synaptic charge in hippocampal neurons [2]. TLQP-62 could be generated by PC1/3 [86]. On the other hand HHPD-41 would represent the proteolytic residue of the TLQP-21's cleavage from TLQP-62 (La Corte et al., unpublished). Our preliminary observations on TLQP-62 and HHPD-41 showed that, unlike TLQP-21, they both possess a positive role on feeding (Rizzi et al., unpublished). In both experiments, mice were overnight fasted, injected with TLQP-62 or HHPD-41 and re-fed. Food intake was monitored for the following 24 h. Results showed that both peptide at doses comprised between 1 and 4 mM determined an increase in food intake up to 24 h following a single icv injection. Further detailed studies are needed before a conclusion can be reached, however, the evidences we collected would suggest that following PC1/3 processing of ProVGF and production of TLQP-62 a further proteolytic processing would produce at least two nutrionally/metabolic active peptides at the C and N terminus of TLQP-62 namely HHPD-41 (or in alternative its internal AQEE-30) and TLQP-21. These two neuropeptides would have an opposite effect on feeding and metabolic functions being anabolic and catabolic, respectively. When comparing our findings with TLQP-21 [11] with the phenotype of VGF-/-, it can be suggested that the endogenous physiological role of TLQP-21 should not be as powerful in opposing the role of other VGF-derived peptides such as HHPD-41 (or AQEE-30).

Future directions

Following the identification of the *vgf* gene [48], the generation of VGF deficient mice [33] and the identification of the first metabolically active VGF peptide TLQP-21 [11] it is now clear that the *vgf* gene is a key regulator of energy homeostasis and the autonomic nervous system. However major challenges are open for further investigations. Among the most important is the clarification of our hypothesis of the existence of at least a second metabolically active VGF-derived peptide. In addition identification of the receptor(s) for VGF peptides and the clarification of their regional distribution within the CNS will open new avenue of research and will provide an invaluable tool for biomedical research and development of new metabolically active drugs

Update added in proof

In the time comprised between acceptance and publication there have been a number of significant advancements on VGF biochemistry, histology and physiology, which are discussed below.

Identification of VGF-derived peptides

Previous reports described several fragments derived from the amino-terminal region of the ProVGF peptide in human cerebrospinal fluids and its association with neurological disorders [62, 80, 95]. Recent reports extend this observation to patients prodromal for or at first-onset psychosis [37, 38] as well as to patients diagnosed as schizophrenia [91]. In addition, two previously unrecognised amidated VGF-derived peptides, secreted from human medullary thyroid carcinoma TT cells, were identified and named NERP-1 and NERP-2 [93]. NERP-1 and NERP-2 correspond to fragments VGF285–VGF311 and VGF314–VGF350 of the rat ProVGF peptide, respectively. Experimental evidence proves that the two peptides dose-dependently suppress vasopressin release induced by NaCl or angiotensin II in vivo and also vasopressin secretion from hypothalamic explants in vitro.

Identification of VGF fragments immunoreactivity in endocrine tissues

Histological evidences now demonstrate that: 1) proVGF-related peptides are present in electron-dense granules within endocrine cells (thyroid, parathyroid, lung, and stomach) early during development and adulthood and increase in hyperplasia and tumors [67]; 2) VGF(556–565) and VGF(282–291) immunoreactivity has been described in delta somatostatin-producing cells, whereas the human C-terminus antiserum selectively immunolabeled alpha glucagon and pancreatic polypeptide cells. The same cells showed immuno-reactivity for VGF(443–588) antiserum

while VGF(298–306) and C-terminus immunoreactivity were found in virtually all pancreatic endocrine cells [21]. Of main interest, Cocco et al. [21] showed that the VGF(556–565) antibody also recognized a number of low molecular mass fractions including a form corresponding to the rat TLQP-21 [11].

Metabolic role of TLQP-21

We have recently extended our observations to TLQP-21: 1) We determined physiological, biochemical and molecular changes associated with diet-induced obesity in a population of fast weight gaining mice (Bartolomucci et al., submitted). Our results demonstrated that chronic icv infusion of TLQP-21 prevents diet-induced obesity despite overfeeding associated with the palatable diet and that these effects are paralleled by activation of catabolic pathways within the eWAT but not within the BAT; 2) we demonstrated that chronic icv TLQP-21 treatment does not modulate the GH-IGF1 axis in adult mice [12].

Following our identification of the central catabolic role of TLQP-21 [11], Jethwa et al. [40] provided evidences that acute icv but not ip administration of TLQP-21 decreased food intake. Chronic icv treatment (daily injection) caused a sustained reduction in food intake and body weight and decreased abdominal fat deposits. No change in energy expenditure was observed. In addition, chronic TLQP-21 did not exert any change in hypothalamic gene mRNA, while determining a reduction in BAT UCP1. Overall these data largely confirm a catabolic role for TLQP-21 which is independent of hypothalamic neuropeptides investigated [11, Bartolomucci et al., submitted]. Therefore, three independent studies [11, 40, Bartolomucci et al., submitted] proved a catabolic role for TLQP-21 but differ in the possible mechanism underlying the effect observed: i) increased energy expenditure/WAT catabolic effects in our studies; ii) reduced food intake in the hamster studies. A number of methodological (repeated injections vs. chronic infusion; the dose used; etc.) and species-specific (hamster have a peculiar physiological adaptation to food-shortage/short day length-induced hibernation [54]) issues may be advocated and should be experimentally ruled out before any conclusion can be drawn on TLQP-21 mechanism of action. Notwithstanding, the conclusion that TLQP-21 negatively affects energy balance and does have a catabolic effect in rodents is now proved by different studies.

Acknowledgments Thanks are due to G. La Corte, V. Locatelli, A. E. Rigamonti, A. Torsello, E. Bresciani, I. Bulgarelli, R. Rizzi, F. R. D'Amato, C. Severini, G. Mignogna, A. Giorgi, M. E. Schininà, G. Elia, C. Brancia, G. -L. Ferri, R. Conti, B. Ciani, T. Pascucci and E. E. Muller, for experimental and theoretical support to the realization of the research described in the review. Supported by FIRB RBNE01JKLF_004 to Anna Moles; FIRB RBNE01JKLF_006, FIRB RBNE013XSJ_004 and IZS SA 002/02 to Roberta Possenti; and FIRS Neurobiotecnologie and Telethon E0830 to Andrea Levi.

References

1. Adage T, Scheurink AJ, de Boer SF, de Vries K, Konsman JP, Kuipers F, Adan RA, Baskin DG, Schwartz MW, van Dijk G (2001) Hypothalamic, metabolic, and behavioral responses to pharmacological inhibition of CNS melanocortin signaling in rats. J Neurosci 21:3639–3645
2. Alder J, Thakker-Varia S, Bangasser DA, Kuroiwa M, Plummer MR, Shors TJ, Black IB (2003) Brain-derived neurotrophic factor-induced gene expression reveals novel actions of vgf in hippocampal synaptic plasticity. J Neurosci 23:10800–10808
3. Amir S, Schiavetto A (1990) Injection of prostaglandin E2 into the anterior hypothalamic preoptic area activates brown adipose tissue thermogenesis in the rat. Brain Res 528:138–142
4. Bachman ES, Dhillon H, Zhang CY, Cinti S, Bianco AC, Kobilka BK, Lowell BB (2002) beta-AR signaling required for diet-induced thermogenesis and obesity resistance. Science 297:843–845
5. Badman MK, Flier JS (2005) The gut and energy balance: visceral allies in the obesity wars. Science 307:1909–1914
6. Baile CA, McLaughlin CL, Della-Fera MA (1986) Role of cholecystokinin and opioid peptides in control of food intake. Physiol Rev 66:172–234
7. Balthasar N, Dalgaard LT, Lee CE, Yu J, Funahashi H, Williams T, Ferreira M, Tang V, McGovern RA, Kenny CD, Christiansen LM, Edelstein E, Choi B, Boss O, Aschkenasi C, Zhang C, Mountjoy K, Kishi T, Elmquist JK, Lowell BB (2005) Divergence of melanocortin pathaways in the control of food intake and energy expenditure. Cell 123:493–505
8. 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
9. Barsh GS, Ollmann MM, Wilson BD, Miller KA, Gunn TM (1999) Molecular pharmacology of agouti protein in vitro and in vivo. Ann NY Acad Sci 885:143–152
10. Bartness TJ, Bamshad M (1998) Innervation of mammalian white adipose tissue: implications for the regulation of total body fat. Am J Physiol 275:R1399–R1411
11. 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, Schininà ME, Elia AG, Brancia C, Ferri G-L, 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
12. Bartolomucci A, Rigamonti AE, Bulgarelli I, Torsello A, Locatelli V, Pavone F, Levi A, Possenti R, Muller EE, Moles A (2007) Chronic intracerebroventricular TLQP-21 delivery does not modulate the GH/IGF-1-axis and muscle strength in mice. Growth Horm IGF Res 17:342–345
13. Beck B (2001) KO's and organization of peptidergic feeding behavior mechanisms. Neurosci Biobehav Rev 25:143–158
14. Bergen HT, Mizuno TM, Taylor J, Mobbs CV (1998) Hyperphagia and weight gain after gold-thioglucose: relation to hypothalamic neuropeptide Y and proopiomelanocortin. Endocrinology 139:4483–4488

15. Bowers RR, Festuccia WT, Song CK, Shi H, Migliorini RH, Bartness TJ (2004) Sympathetic innervation of white adipose tissue and its regulation of fat cell number. Am J Physiol Regul Integr Comp Physiol 286:R1167–R1175
16. Cannon B, Nedegaard J (2004) Brown adipose tissue: function and physiological significance. Physiol Rev 84:277–359
17. Castanon N, Scearce-Levie K, Lucas JJ, Rocha B, Hen R (2000) Modulation of the effects of cocaine by 5-HT1B receptors: a comparison of knockouts and antagonists. Pharmacol Biochem Behav 67:559–566
18. Cederberg A, Grønning LM, Ahren B, Tasken K, Carlsson P, Enerback S (2001) FOXC2 is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance. Cell 106:563–573
19. Chakraborty TR, Tkalych O, Nanno D, Garcia AL, Devi LA, Salton SR (2006) Quantification of VGF- and pro-SAAS-derived peptides in endocrine tissues and the brain, and their regulation by diet and cold stress. Brain Res 1089:21–32
20. Cinti S (2005) The adipose organ. Prostaglandins Leukot Essent Fatty Acids 73:9–15
21. Cocco C, Brancia C, Pirisi I, D'Amato F, Noli B, Possenti R, Ferri GL (2007) VGF metabolic-related gene: distribution of its derived peptides in mammalian pancreatic islets. J Histochem Cytochem 55:619–628
22. Cone RD (2005) Anatomy and regulation of the central melanocortin system. Nat Neurosci 8:571–578
23. Elliott JC, Harrold JA, Brodin P, Enquist K, Backman A, Bystrom M, Lindgren K, King P, Williams G (2004) Increases in melanin-concentrating hormone and MCH receptor levels in the hypothalamus of dietary-obese rats. Mol Brain Res 128:150–159
24. Evans RM, Barish GD, Wang Y-X (2004) PPARs and the complex journey to obesity. Nat Med 10:1–7
25. Ferri GL, Levi A, Possenti R (1992) A novel neuroendocrine gene product: selective VGF8a gene expression and immunolocalisation of the VGF protein in endocrine and neuronal populations. Brain Res Mol Brain Res 13:139–143
26. Flier JS (2004) Obesity wars: molecular progress confronts an expanding epidemic. Cell 116:337–350
27. Fliers E, Kreier F, Voshol PJ, Havekes LM, Sauerwein HP, Kalsbeek A, Buijs RM, Romijn JA (2003) White adipose tissue: getting nervous. J Neuroendocrinol 15:1005–1010
28. Friedman JM, Halaas JL (1998) Leptin and the regulation of body weight in mammals. Nature 395:763–770
29. Friedman JM (2004) Modern science versus the stigma of obesity. Nat Med 10:563–569
30. Garcia AL, Han SK, Janssen WG, Khaing ZZ, Ito T, Glucksman MJ, Benson DL, Salton SR (2005) A prohormone convertase cleavage site within a predicted alpha-helix mediates sorting of the neuronal and endocrine polypeptide VGF into the regulated secretory pathway. J Biol Chem 280:41595–41608
31. Gerlai R (1996) Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype? Trends Neurosci 19:177–181
32. Gingrich JA, Hen R (2000) The broken mouse: the role of development, plasticity and environment in the interpretation of phenotipic changes in knockout mice. Curr Opin Neurobiol 10:146–152
33. 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 SRJ (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
34. Hahm S, Fekete C, Mizuno TM, Windsor J, Yan H, Boozer CN, Lee C, Elmquist JK, Lechan RM, Mobbs CV, Salton SRJ (2002) VGF is required for obesity induced by diet, gold thioglucose treatment and agouti, and is differentially regulated in POMC- and NPY-containing arcuate neurons in response to fasting. J Neurosci 22:6929–6938
35. Heleniak EP, Aston B (1989) Prostaglandins, brown fat and weight loss. Med Hypotheses 28:13–33
36. Horvath TL (2005) The hardship of obesity: a soft-wired hypothalamus. Nat Neurosci 8:561–565
37. Huang JT, Leweke FM, Oxley D, Wang L, Harris N, Koethe D, Gerth CW, Nolden BM, Gross S, Schreiber D, Reed B, Bahn S (2006) Disease biomarkers in cerebrospinal fluid of patients with firstonset psychosis. PLoS Med 3:e428
38. Huang JT, Leweke FM, Tsang TM, Koethe D, Kranaster L, Gerth CW, Gross S, Schreiber D, Ruhrmann S, Schultze-Lutter F, Klosterkotter J, Holmes E, Bahn S (2007) CSF metabolic and proteomic profiles in patients prodromal for psychosis. PLoS ONE 2:e756
39. Jethwa PH, Warner A, Brameld JM, Keyte JW, Nilaweera NK, Morgan PJ, Barrett P, Ebling FJP (2006) The role of a VGF derived peptide in the regulation of food intake in a seasonal rodent. Front Neuroendocrinol 27:5–6
40. Jethwa PH, Warner A, Nilaweera KN, Brameld JM, Keyte JW, Carter WG, Bolton N, Bruggraber M, Morgan PJ, Barrett P, Ebling FJ (2007) VGF-derived peptide, TLQP-21, regulates food intake and body weight in Siberian hamsters. Endocrinology 148:4044–4055
41. Jimenez M, Leger B, Canola K, Lehr L, Arboit P, Seydoux J, Russell AP, Giacobino JP, Muzzin P, Preitner F (2002) β1/(β2/(β3-adrenoreceptor knockout mice are obese and cold sensitive but have normal lipolytic responses to fasting. FEBS Lett 530:37–40
42. Kaput J, Astley S, Renkema M, Ordovas J, van Ommen B (2006) Harnessing nutrigenomics: development of web-based communication, databases, resources, and tools. Genes Nutr 1:5–12
43. Konsman JP, Parnet P, Dantzer R (2002) Cytokines-induced sickness behavior: mechanisms and implications. Trends Neurosci 25:154–159
44. Kranenburg O, Gent YY, Romijn EP, Voest EE, Heck AJ, Gebbink MF (2005) Amyloid-beta-stimulated plasminogen activation by tissue-type plasminogen activator results in processing of neuroendocrine factors. Neuroscience 131:877–886
45. Lam TK, Schwartz GJ, Rossetti L (2005) Hypothalamic sensing of fatty acids. Nat Neurosci 8:579–584
46. Larsen TM, Toubro S, van Baak MA, Gottesdiener KM, Larson P, Saris WHM, Astrup A (2002) Effect of a 28-d treatment with L-796568, a novel β3-adrenergic receptor agonist, on energy expenditure and body composition in obese men. Am J Clin Nutr 76:780–788
47. Leibowitz SF, Wortley KE (2004) Hypothalamic control of energy balance: different peptides, different functions. Peptides 25:473–504
48. Levi A, Eldridge JD, Paterson BM (1985) Molecular cloning of a gene sequence regulated by nerve growth factor. Science 229:393–395
49. Levi A, Ferri GL, Watson E, Possenti R, Salton SR (2004) Processing, distribution, and function of VGF, a neuronal and endocrine peptide precursor. Cell Mol Neurobiol 24:517–533
50. Lim J, Berezniuk I, Che F-Y, Parikh R, Biswas R, Pan H, Fricker LD (2006) Altered neuropeptide processing in prefrontal cortex of $Cpe^{fat/fat}$ mice: implications for neuropeptide discovery. J Neurochem 96:1169–1181
51. Liu JW, Andrews PC, Mershon JL, Yan C, Allen DL, Ben-Jonathan N (1994) Peptide V: a VGF-derived neuropeptide purified from bovine posterior pituitary. Endocrinology 135: 2742–2748
52. Lowell BB, Flier JS (1997) Brown adipose tissue, β3-adrenergic receptors, and obesity. Annu Rev Med 48:307–316

53. Lowell BB, Spiegelman BM (2000) Toward a molecular understanding of adaptive thermogenesis. Nature 404:652–660
54. Morgan PJ, Ross AW, Mercer JG, Barrett P (2006) What can we learn from seasonal animals about the regulation of energy balance? Prog Brain Res 153:325–337
55. Morris MJ, Tortelli CF, Filippis A, Proietto J (1998) Reduced BAT function as a mechanism for obesity in the hypophagic, neuropeptide Y deficient monosodium glutamate-treated rat. Regul Pept 25:441–447
56. Netea MG, Joosten LA, Lewis E, Jensen DR, Voshol PJ, Kullberg BJ, Tack CJ, van Krieken H, Kim SH, Stalenhoef AF, van de Loo FA, Verschueren I, Pulawa L, Akira S, Eckel RH, Dinarello CA, van den Berg W, van der Meer JW (2006) Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. Nat Med 12:650–656
57. Nogueiras R, Tovar S, Mitchell SE, Rayner DV, Archer ZA, Dieguez C, Williams LM (2004) Regulation of hormone segretagogue receptor gene expression in the arcuate nuclei of the rat by leptin and ghrelin. Diabetes 53:2552–2558
58. Nonogaki K (2000) New insights into sympathetic regulation of glucose and fat metabolism. Diabetologia 43:533–549
59. Obici S, Feng Z, Arduini A, Conti R, Rossetti L (2003) Inhibition of hypothalamic carnitine palmitoyltransferase-1 decreases food intake and glucose production. Nat Med 9:56–61
60. Orci L, Cook WS, Ravazzola M, Wang MY, Park BH, Montesano R, Unger RH (2004) Rapid transformation of white adipocytes into fat-oxidizing machines. Proc Natl Acad Sci USA 101:2058–2063
61. Pan H, Nanno D, Che F-H, Zhu X, Salton SR, Steiner DF, Fricker LD, Devi LA (2005) Neuropeptide processing profile in mice lacking prohormone convertase-1. Biochemistry 44:4939–4948
62. Pasinetti GM, Ungar LH, Lange DJ, Yemul S, Deng H, Yuan X, Brown RH, Cudkowicz ME, Newhall K, Peskind E, Marcus S, Ho L (2006) Identification of potential CSF biomarkers in ALS. Neurology 66:1218–1222
63. Pizzi WJ, Barnhart JE (1976) Effects of monosodium glutamate on somatic development, obesity and activity in the mouse. Pharmacol Biochem Behav 5:551–557
64. Possenti R, Eldridge JD, Paterson BM, Grasso A, Levi A (1989) A protein-induced by NGF in PC12 cells is stored in secretory vescicles and released through the regulated pathway. EMBO J 8:2217–2223
65. 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 140:3727–3235
66. Rayner DV (2001) The sympathetic nervous system in white adipose tissue regulation. Proc Nutr Soc 60:357–364
67. Rindi G, Licini L, Necchi V, Bottarelli L, Campanini N, Azzoni C, Favret M, Giordano G, D'Amato F, Brancia C, Solcia E, Ferri GL (2007) Peptide products of the neurotrophin-inducible gene vgf are produced in human neuroendocrine cells from early development and increase in hyperplasia and neoplasia. J Clin Endocrinol Metab 92:2811–2815
68. Riu E, Ferre T, Hidalgo A, Mas A, Franckhauser S, Otaegui P, Bosch F (2003) Overexpression of c-myc in the liver prevents obesity and insulin resistance. FASEB J 17:1715–1717
69. Robinson SW, Dinulescu DM, Cone RD (2000) Genetic models of obesity and energy balance in the mouse. Annu Rev Genet 34:687–745
70. Rocha BA, Scearce-Levie K, Lucas JJ, Hiroi N, Castanon N, Crabbe JC, Nestler EJ, Hen R (1998) Increased vulnerability to cocaine in mice lacking the serotonin-1B receptor. Nature 393:175–178
71. Ross AW, Bell LM, Littlewood PA, Mercer JG, Barrett P, Morgan PJ (2005) Temporal changes in gene expression in the arcuate nucleus precede seasonal responses in adiposity and reproduction. Endocrinology 146:1940–1947
72. Rossi A, Granata F, Augusti-Tocco G, Canu N, Levi A, Possenti R (1992) Expression in murine and human neuroblastoma cell lines of VGF, a tissue specific protein. Int J Dev Neurosci 10:527–534
73. Sahu A (2003) Leptin signaling in the hypothalamus: emphasis on energy homeostasis and leptin resistance. Front Neuroendocrinol 24:225–253
74. Salton SRJ, Fischberg DJ, Dong K-W (1991) Structure of the gene encoding VGF, a nervous system-specific mRNA that is rapidly and selectively induced by nerve growth factor in PC12 cells. Mol Cell Biol 11:2335–2349
75. Salton SR, Ferri GL, Hahm S, Snyder SE, Wilson AJ, Possenti R, Levi A (2000a) VGF: a novel role for this neuronal and neuroendocrine polipeptide in the regulation of energy balance. Front Neuroendocrinol 21:199–219
76. Salton SR, Hahm S, Mizuno TM (2000b) Of mice and MEN: what transgenic models tell us about hypothalamic control of energy balance. Neuron 25:265–268
77. Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG (2000) Central nervous system control of food intake. Nature 404:661–671
78. Snyder SE, Cheng HW, Murray KD, Isackson PJ, McNeill TH, Salton SR (1998) The messenger RNA encoding VGF, a neuronal peptide precursor, is rapidly regulated in the rat central nervous system by neuronal activity, seizure and lesion. Neuroscience 82:7–19
79. Song CK, Jackson RM, Harris RBS, Richard D, Bartness TJ (2005) Melanocortin-4 receptor mRNA is expressed in sympathetic nervous system outflow neurons to white adipose tissue. Am J Physiol Regul Integr Comp Physiol 289:R1467–R1476
80. Stark M, Danielsson O, Griffiths WJ, Jornvall H, Johansson J (2001) Peptide repertoire of human cerebrospinal fluid: novel proteolytic fragments of neuroendocrine proteins. J Chromatogr B Biomed Sci Appl 754:357–367
81. Strubbe JH, van Dijk G (2002) The temporal organization of ingestive behaviour and its interaction with regulation of energy balance. Neurosci Biobehav Rev 26:485–498
82. Succu S, Cocco C, Mascia MS, Melis T, Melis MR, Possenti R, Levi A, Ferri GL, Argiolas A (2004) Pro-VGF-derived peptides induce penile erection in male rats: possible involvement of oxytocin. Eur J Neurosci 20:3035–3040
83. Succu S, Mascia MS, Melis T, Sanna F, Melis MR, Possenti R, Argiolas A (2005) Pro-VGF-derived peptides induce penile erection in male rats: involvement of paraventricular nitric oxide. Neuropharmacology 49:1017–1025
84. Tiraby C, Langin D (2003) Conversion from white to brown adipocytes: a strategy for the control of fat mass. Trends Endocrinol Metab 14:439–441
85. 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
86. Trani E, Giorni 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
87. Uyama N, Geerts A, Reynaert H (2004) Neural connections between the hypothalamus and the liver. Anat Rec Discov Mol Cell Evol Biol 280:808–820
88. 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
89. Wang Y, Lee C-H, Tiep S, Yu RT, Ham J, Kang H, Evans RM (2003) Peroxisome-proliferator-activated receptor δ activates fat metabolism to prevent obesity. Cell 113:159–170

90. 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
91. Wedenoja J, Loukola A, Tuulio-Henriksson A, Paunio T, Ekelund J, Silander K, Varilo T, Heikkila K, Suvisaari J, Partonen T, Lonnqvist J, Peltonen L (2007) Replication of linkage on chromosome 7q22 and association of the regional Reelin gene with working memory in schizophrenia families. Mol Psychiatry (in press)
92. Wisor JP, Takahashi JS (1997) Regulation of the vgf gene in the golden hamster suprachiasmatic nucleus by light and by the circadian clock. J Comp Neurol 378:229–238
93. Yamaguchi H, Sasaki K, Satomi Y, Shimbara T, Kageyama H, Mondal MS, Toshinai K, Date Y, Gonzalez LJ, Shioda S, Takao T, Nakazato M, Minamino N (2006) Peptidomic identification and biological validation of neuroendocrine regulatory Peptide-1 and -2. J Biol Chem 282:26354–26360
94. Yaswen L, Diehl N, Brennan MB, Hochgeschwender U (1999) Obesity in the mouse model of pro-opiomelanocortin deficiency responds to peripheral melanocortin. Nat Med 5:1066–1070
95. Yuan X, Desiderio DM (2005) Human cerebrospinal fluid peptidomics. J Mass Spectrom 40:176–181
96. Zhang JV, Ren PG, Avsian-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
97. Zigman JM, Nakano Y, Coppari R, Balthasar N, Marcus JN, Lee CE, Jones JE, Deysher AE, Waxman AR, White RD, Williams TD, Lachey JL, Seeley RJ, Lowell BB, Elmquist JK (2006) Mice lacking ghrelin receptors resist the development of diet induced obesity. J Clin Invest 115:3564–3572
98. Ziotopulou M, Mantzoros CS, Hileman SM, Flier JS (2000) Differential expression of hypothalamic neuropeptides in the early phase of diet-induced obesity in mice. Am J Physiol Endocrinol Metab 279:E838–E845