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RESEARCH PAPER

Chronic intracerebroventricular injection of TLQP-21 prevents high fat diet induced weight gain in fast weight-gaining mice

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Abstract The *vgf* gene regulates energy homeostasis and the VGF-derived peptide TLQP-21 centrally exerts catabolic effects in mice and hamsters. Here, we investigate the effect of chronic intracerebroventricular (icv) injection of TLQP-21 in mice fed high fat diet (HFD). Fast weight-gaining mice injected with the peptide or cerebrospinal fluid were selected for physiological, endocrine, and molecular analysis. TLQP-21 selectively inhibited the increase in body weight and epididymal white adipose tissue (eWAT) weight induced by HFD in control animals despite both groups having a similar degree of hyperphagia. TLQP-21 normalized the increase in leptin and decrease in ghrelin while increasing epinephrine and epinephrine/norepinephrine ratio when compared to values in controls. Finally, HFD-TLQP-21 mice showed a selective increase of eWAT β3-adrenergic receptor mRNA. Peroxisome-proliferator-activated-receptor-δ and hormone-sensing-lipase mRNA were also upregulated. In conclusion, chronic icv infusion of TLQP-21 prevented the early phase of diet-induced obesity despite overfeeding. These effects were paralleled by activation of catabolic pathways within the eWAT. Our results further support a role for TLQP-21 as a catabolic neuropeptide.

Keywords VGF · Obesity · Neuropeptides · White adipose tissue · High fat diet

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Introduction

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 [34, 48, 62]. In particular, catabolic fasting/energy dissipating pathways downstream of hypothalamic nuclei projects to other brain area or the pituitary and leads to the coordinated activation of the sympathetic innervations to metabolic tissues, the release of epinephrine from the adrenal medulla, the thyroid axis, behavioral energy-dissipating activities as well as inhibition of feeding. Synergistic activation of these pathways finally leads to increased energy expenditure and dissipation (rise in body temperature), as well as lipolysis [34, 41, 62]. Peripheral target tissues of these pathways

include metabolic tissues such as muscles, liver, as well as the brown adipose tissue (BAT) and the white adipose tissue (WAT) [14, 18, 52, 67].

In this context, the number of genes involved in the regulation of nutrition and metabolism in general, and catabolic energy dissipating pathways in particular, is enormous [6, 60] and the identification of peptides associated with higher or lower risk for obesity is rising exponentially [29, 30]. Among these genes, *vgf* [42] is gaining increasing interest. VGF mRNA can be upregulated by fasting in the arcuate nucleus (ARC) with leptin limiting the fasting-induced increase [33]. VGF co-localizes in the ARC with proopiomelanocortin (POMC) and modestly with peuropeptide Y (NPY) expressing neurons in the fed state. Co-localization increased with NPY and decreased with POMC in the fast state [33]. In addition, germline VGF−/− mice have a lean, hypermetabolic, and obesity resistant phenotype [32, 33]. However, the *vgf* gene encodes a phylogenetically highly conserved precursor protein of 615 (human) and 617 (rat, mice) amino acids [43, 61]. 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. 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 regulated pathways [66]. Up to now, six VGF-peptides were shown to modulate with a remarkable peptide-specific selectivity, a diverse range of biological functions such as synaptic plasticity, apoptosis, sexual antidepressant-like behavior and inflammatory pain [2, 7, 10, 11, 35, 37, 58, 63, 64, 65, 70].

Among the different VGF-peptides, however, only the peptide designate TLQP-21 was demonstrated to exert a catabolic role [7, 37], while preliminary data showed an orexigenic effect following acute injection of the peptides designated TLQP-62 and HHPD-41 [8]. In particular, Bartolomucci et al. [7] proved that chronic icv injection of TLQP-21 increased resting energy expenditure and prevented the early phase of diet-induced obesity and associated leptin and ghrelin alterations. A later study by Jethwa et al. [36, 37] demonstrated in hamster an anorectic effect exerted by TLQP-21 which determined a decrease in body weight and adipose fat mass.

In the present study, we aimed at extending current knowledge on TLQP-21 in high fat fed mice. High fat feeding can affect a variety of brain and peripheral pathways involved in energy balance [25, 26] including beta adrenergic function [21]. Furthermore, these effects are dependent upon genetic vulnerability to diet-induced obesity and are correlated with lipid and glucose metabolism [39, 46]. To test if the effects of TLQP-21 could be related to the mice liability to weight gain, TLQP-21 was chronically injected icv in mice fed high fat diet, and post hoc selected as fast weight gaining within each experimental group. Physiological parameters, metabolic-regulating hormones as well as molecular changes in the adipose tissue were analyzed.

Materials and methods

Animals

Male Swiss CD1 mice weighting 30–35 g were purchased from Charles River (Calco, Lecco, Italy) and housed in groups of four in an environmentally controlled room (temperature, 20–22°C; Light on 07:00, off 19:00). Mice were allowed 15 days to acclimatize to the laboratory conditions. Food (3.4 Kcal/g, Mucedola s.r.l.) and water were available ad libitum. All experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

Peptide

Synthetic TLQP-21 peptide [7] (Primm, Milano, Italy) at the dose of 1 mM dissolved in artificial cerebrospinal fluid (aCSF) or aCSF alone was icv delivered trough Alzet micro-osmotic pumps (Mod. 1002; final volume of 100 µl; flow rate 0.25 µl/h, corresponding to 15 µg/day of TLQP-21) connected to the Alzet brain infusion kit. The dose was selected based on previous studies [7–10]. To allow preloading, pumps were filled the day before surgery and overnight incubated in sterile saline at 37°C.

Experimental paradigm

All experiments consisted of a 4-day baseline period followed by a 14-day experimental phase in which mice were fed a standard chow and treated aCSF ($n = 9$) or received a high fat diet consisting of diluted powdered rodent chow plus 20% lard (calculated energy content of the dry diet 4.33 Kcal/g) and were treated with aCSF ($n = 12$) or TLQP-21 ($n = 11$).

Along the whole experimental phase, animals were individually housed and locomotor activity was monitored continuously (see below). Body weight was determined with an analytical balance (precision level 0.1 g) on day 0 (days of surgery, referred to as BASAL), day 1 and every second day. Food intake was monitored on a daily basis. Animals were terminated by decapitation, following brief

CO_2 exposure, on day 14 between 9:00 and 11:00 am. At autopsy, interscapular brown adipose tissue (iBAT) and epididymal white adipose tissue (eWAT) were dissected out, weighted, and snap frozen in liquid nitrogen.

Surgery

Alzet pumps and brain infusion kits were implanted under anesthesia (ketamine, 100 mg/kg, ip and xylazine, 5 mg/kg, ip). The mouse head was fixed in a stereotaxic apparatus and an incision was made in the atlanto-occipital membrane. Cannulae were implanted according to the stereotaxic co-ordinates (anteroposterior, AP −0.1 mm; mediolateral, ML ± 1.0 mm; dorsoventral, DV 3 mm form bregma) derived from the mouse brain atlas [28]. Cannulae were fixed to the mouse skull with polycarbonate cement (Hy-Bond Polycarbonate Cement, Shofu Inc, Kyoto, Japan). Localization of the cannulae was confirmed by icv injection of colorant in a subgroup of animals.

Home cage locomotor activity

The assessment of daily activity was carried out by means of an automated system that used small passive infrared sensors positioned on the top of each cage (Activiscope, New Behaviour Inc., Zürich, Switzerland).

Serum measurements

Mouse serum ghrelin was measured by a commercial radioimmunoassay (RIA) kit (Linco Research, Inc, MO, USA). Sensitivity of the assay is 93 pg/ml. The inter-assay coefficient of variation (CV) was 9.0%, and the intra-assay CV was 3.3%. Serum leptin was measured by enzyme-linked immunosorbent assay (ELISA) (Linco Research, Inc, MO, USA). Sensitivity of the assay is 0.5 ng/ml, and the inter- and intra-assay CV were 5.7 and 2.0%, respectively. Norepinephrine (NE) and epinephrine (E) levels were determined simultaneously using an HPLC system (Alliance, Waters) coupled with a coulometric detector (Model 5200A Coulochem II, ESA) provided with a 5011 high sensitivity analytical cell and 5021 conditioning cell. The potentials were set at +450 and +100 mV at the analytical and the conditioning cell, respectively. The columns, a Nova-Pack Phenyl column (3.9 × 150 mm) and a Sentry Guard Nova-Pack precolumn (3.9 × 20 mm), were purchased from Waters Corporation. The flow rate was 1.2 ml/min. The mobile phase consisted of 3% methanol in 0.1-M Na–phosphate buffer, pH 3, 0.1 mM Na_2EDTA, and 0.5-mM 1-octane sulfonic acid Na salt (Sigma). For catecholamines determination, serum was treated with $HClO_4$ 3.4 M with a ratio of 1 µl serum to 50 µl $HClO_4$.

RT-PCR

BAT and WAT were quickly dissected, collected in RNA-later (Sigma-Aldrich, St. Louis, MO, USA) and subsequently frozen and stored at −20°C until processed for RNA extraction. Total RNA was extracted from cells using TRI-zol-like reagent; it is an improvement to the single-step RNA isolation method developed by Chomczynski and Sacchi [17]. RNA quality was assessed with the NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies) (260/280 and 260/230 nm ratios) and the integrity of extracted RNA was examined by electrophoresis. Three thousands nanogram of total RNA were incubated with rDNase I (Ambion) for 20 min at 37°C to digest contaminating genomic DNA. Two hundred nanogram total RNA of each sample were subjected to reverse transcription with Moloney murine leukemia virus reverse transcriptase (Invitrogen, Carlsbad, CA, USA) followed by amplification with TAQ (GoTAQ, Promega, Madison, WI, USA) using specific primers based on the published sequence of peroxisome proliferator activated receptor (PPAR)-δ, β1-adrenergic receptor (AR), β2-AR, β3-AR, uncoupling protein (UCP)-1, UCP-2, UCP-3, hormone sensing lipase (HSL) (Table 1). Semiquantitative PCR analysis of total RNA yielded a DNA fragment of the expected length for all specific mRNAs. All samples were assayed in triplicate. The band density was analyzed with Kodak 1D Image Analysis Software. To normalize results for differences in RNA sampling, an aliquot of the same RT reaction was used to amplify a glyceraldehyde-6-phosphate (GADPH) 600-bp fragment (Table 1). To assure that PCR was performed in the linear amplification range, samples were initially analyzed after 15, 17, 20, 25, 27, 30, 35 and 40 cycles (data not shown). For each factor, we choose the cycle number that gave half of the maximal amplification. Negative controls of the PCR reactions were made omitting the specific primers from the mixture.

Statistical analysis

Data were analyzed with univariate or multivariate (for repeated measures) ANOVA (followed by Tukey's HSD post hoc tests) where appropriate.

Results

TLQP-21 selectively blocks weight gain and adiposity in fast body weight-gaining mice

In a previous report we proved that chronic icv injection of TLQP-21 could prevent diet-induced obesity in mice. However, obesity prone and obesity resistant populations have been described in humans and animal models [3, 6].

Table 1 Nucleotide sequence of primers used in the RT-PCR assay

Gene	Forward primer	Reverse primer	$T\alpha$ (°C)	No. of cycles	Amplicone (bp)
PPAR-δ	GTCGCACAACGCTATCCGCT	CCTTCTCTGCCTGCCACA	61	35 WAT	259
β1-AR	CGTCCGTCGTCTCCTTCTAC	TGATGATGCCCAGTGTCTTG	53	35 BAT	154
				35 WAT	
β2-AR	TGGTGGTGATGGTCTTTGTC	GTCTTGAGGGCTTTGTGCT	58	34 BAT	187
				33 WAT	
β3-AR	GCCGAGACTACAGACCATAACC	CGAGCATAGACGAAGAGCATT	56	34 BAT	483
				30 WAT	
UCP-1	AACAGAAGGATTGCCGAAACT	AATGAACACTGCCACACCTC	61	25 BAT	190
				34 WAT	
UCP-2	CAGTTCTACACCAAGGGCTCAGAG	TCTGTCATGAGGTTGGCTTTCAG	62	34 BAT	320
				34 WAT	
UCP-3	GGAGGAGAGAGGAAATACAGAGG	CCAAAGGCAGAGACAAAGTGA	58	28 BAT	218
				33 WAT	
HSL	GAGATTGAGGTGCTGTCG	TCGTGGGATTTAGAGGTC	55	26 WAT	350
GaPDH	GCCATCAACGACCCCTTCATTG	TGCCAGTGAGCTTCCCGTTC	53	27 BAT	603
				33 WAT	

Mice receiving high fat diet (HFD) were classified as more than or less than average prone towards weight gain. Specifically, mice increasing (prone) or not (resistant) body weight were identified if their body weight change after 14-days HFD treatment had fallen over or below the group median (Fig. 1). Mice prone to weight gain receiving either TLQP-21 (HFD-TLQP-21, $n = 6$) or artificial cerebrospinal fluid (HFD-aCSF, $n = 6$) or mice fed standard diet (SD-aCSF, $n = 9$) were the focus of this study (gray dots in Fig. 1). There was no pre-existing difference in body weight between groups (SD-aCSF, 37 ± 0.7; HFD-aCSF, 35.7 ± 0.9; HFD-TLQP-21, 35.9 ± 0.8; ANOVA, ns).

As expected HFD-aCSF mice were hyperphagic, gained weight, showed high metabolic efficiency and WAT hyperplasia when compared to SD-aCSF mice (Fig. 2; ANOVA main effects: food intake: treatment $F(2,16) = 10.3$, $P < 0.01$; body weight: treatment $F(2,18) = 12.0$, $P < 0.001$; metabolic efficiency, treatment $F(2,17) = 14.3$, $P < 0.01$; WAT, treatment $F(2,18) = 3.86$, $P < 0.05$; see figure for post hoc analysis). TLQP-21 selectively inhibited the increase in body weight, metabolic efficiency and WAT weight induced by high fat diet (Fig. 2). Importantly, these metabolic effects occurred despite HFD-TLQP-21 mice had a similar level of hyperphagia than HFD-aCSF mice (Fig. 2), without any effect on locomotor activity (data not shown).

TLQP-21 also selectively blunted the obesity-associated increase in leptin and decrease in ghrelin shown by HFD-aCSF mice (ANOVA main effects: leptin: $F(4,29) = 3.0$, $P < 0.05$; ghrelin: $F(4,27) = 2.3$, $P < 0.09$; see Fig. 3 for post hoc analysis).

Finally, both HFD treated groups showed a decrease in NE while HFD-TLQP-21 had an increase in E when compared

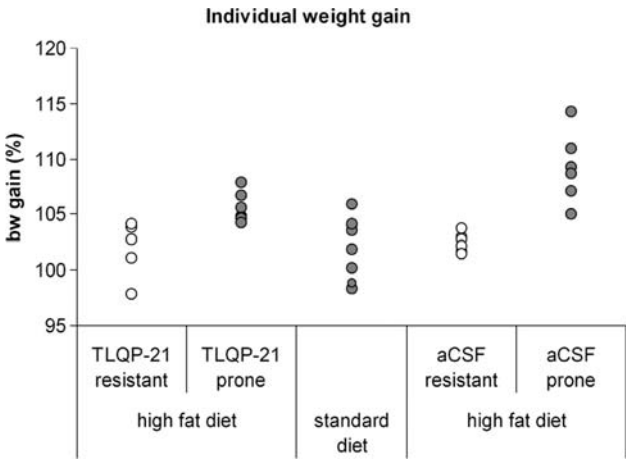

Fig. 1 Selection of mice prone or resistant to weight gain following 14-day high fat diet. Mice were selected to be prone or resistant based on their final body weight gain to fall above or below the median of each experimental group. Individuals identified with a *gray dot* were selected and included in the analyses described in the study

to HFD-aCSF but not to SD-aCSF (Fig. 3; ANOVA main effects: NE, treatment $F(2,16) = 5.8$, $P < 0.05$; E, treatment $F(2,14) = 2.4$, $P = 0.1$; see Fig. 3 for post hoc analysis). In addition, HFD-TLQP-21 mice also had an increased E/NE ratio (ANOVA main effects: treatment $F(2,14) = 3.8$, $P < 0.05$; see figure for post hoc analysis).

TLQP-21 selectively upregulated expression of catabolic markers in the eWAT

HFD-TLQP-21 mice showed (Fig. 4a) a selective increase of β3-AR (ANOVA main effect: treatment $F(2,17) = 5.45$,

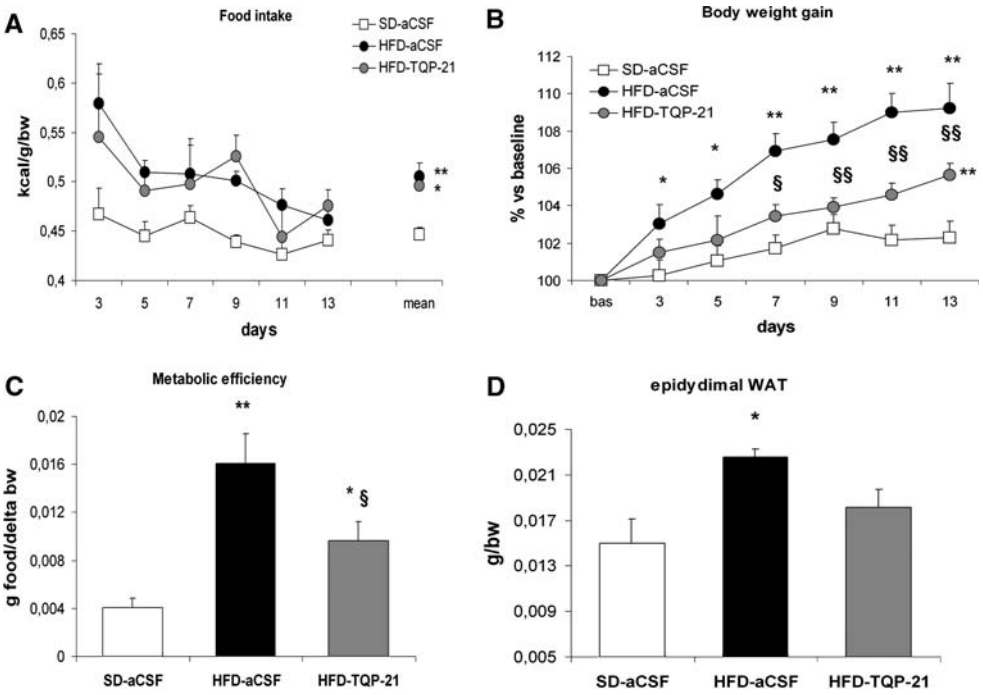

Fig. 2 Metabolic consequences of high fat diet and icv TLQP-21 treatment. The figure shows food intake (**a**), body weight gain (**b**), metabolic efficiency (**c**) and weight of the epididymal white adipose tissue (**d**) in mice fed a standard (SD) or a high fat diet (HFD) and chronically intracerebroventricularly injected with artificial cerebrospinal fluid (aCSF) or TLQP-21. ** and $^{\S\S}P < 0.01$, * and $^{\S}P < 0.05$ versus SD-aCSF and versus HFD-aCSF, respectively

$P < 0.05$) when compared to SD-aCSF and HFD-aCSF mice. In addition, HFD-TLQP-21 mice showed increased expression of PPAR-δ (ANOVA main effect: treatment $F(2,18) = 4.7$, $P < 0.05$) and of HSL (ANOVA main effect: treatment $F(2,18) = 5.4$, $P < 0.05$) when compared to control SD-aCSF mice only. In addition, HFD-TLQP-21 mice also had a slight but not significant increase (post hocs, $P < 0.09$) in eWAT β2-AR and β1-AR when compared to SD-aCSF mice (ANOVA main effects: β2-AR, treatment $F(2,16) = 2.9$ $P < 0.08$; β1-AR, treatment $F(2,17) = 2.9$ $P < 0.08$). Finally, HFD also increased eWAT UCP2 expression in both HFD-TLQP-21 and HFD-aCSF mice when compared to SD-aCSF (ANOVA main effect: treatment $F(2,17) = 6.3$, $P < 0.05$). TLQP-21 had no effect on UCP1 or UCP3 expressed in eWAT (data not shown).

On the other hand, TLQP-21 was not associated with any change in gene expression in the interscapular brown adipose tissue (iBAT; Fig. 4b).

Discussion

In this study, we have demonstrated that chronic icv infusion of the VGF-derived peptide TLQP-21 prevented weight gain and associated endocrine and molecular alterations induced by high fat diet. These findings were obtained in mice post hoc selected as fast weight gaining within each experimental group. Of note, TLQP-21-treated mice despite being resistant to weight gain showed similar hyperphagia than controls. The lack of increase in adipose tissue is the more likely explanation for hormonal findings, i.e., the lack of increase in leptin and the decrease in ghrelin, which were evident instead in HFD-aCSF mice [47, 56, 69]. This study confirms our previous report [7] but partially contrasts the findings by Jethwa et al. [37] who showed that daily icv injection of TLQP-21 exerted a catabolic effect in the Siberian hamster which was associated with decreased body and WAT weight and was dependent on reduced food intake but not on increased energy expenditure. Therefore, three independent studies [7, 37]; present study) evidence a catabolic role for TLQP-21, but differ in the possible mechanism underlying the effect observed: (1) increased energy expenditure/WAT catabolic effects in our studies; (2) 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, see [50]) issues may be advocated and should be experimentally ruled out before a 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 on the adipose organ is emerging as a constant finding and is further strengthened by the present study.

No receptor has been so-far described for TLQP-21 and no reliable biochemical assay has been developed to detect TLQP-21; therefore, we still do not know where the peptide is expressed in the brain and where it exerts its functions (although some recent development has been obtained in peripheral immunoreactivity of VGF-peptides

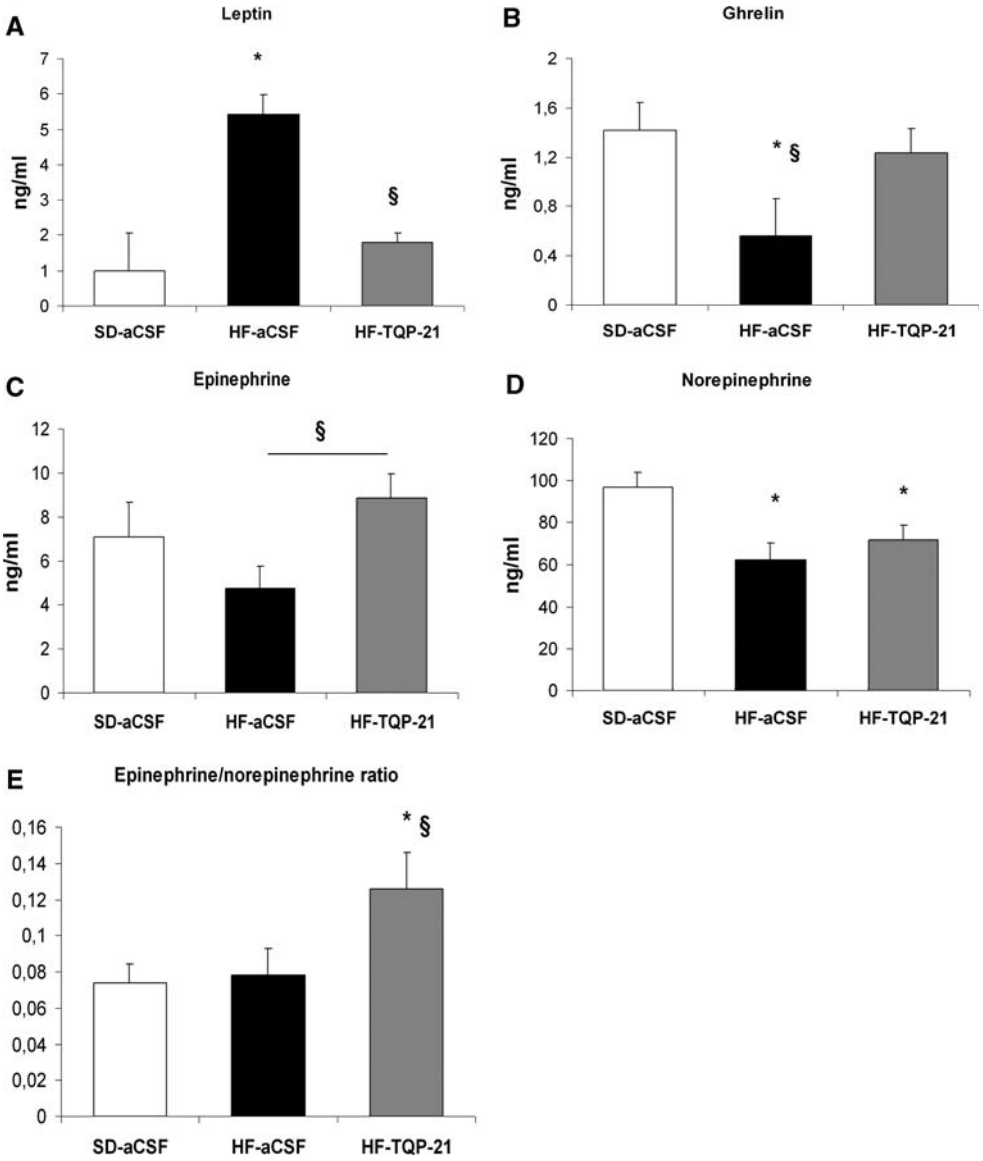

Fig. 3 Endocrine consequences on high fat diet and icv TLQP-21 treatment. The figure shows serum leptin (**a**), ghrelin (**b**), epinephrine (**c**) norepinephrine (**d**) and epinephrine/norepinephrine ratio (**e**) in mice fed a standard (SD) or a high fat diet (HFD) and chronically intracerebroventricularly injected with artificial cerebrospinal fluid (aCSF) or TLQP-21. * and $^{\S}P < 0.05$ versus SD-aCSF and versus HFD-aCSF, respectively

[19, 57] and with other VGF-derived peptides [16]). However, according to the previous studies [7, 37], TLQP-21 exerted its catabolic effects independently of any gene expression change in the hypothalamus thus allowing the conclusion that the site(s) of TLQP-21 activity lies downstream of the hypothalamic circuits.

In agreement with our previous report, resistance to obesity was associated with increased expression of catabolic mediators such as β3-AR (and to a lower extent also β1- and β2-AR) and PPAR-δ in the eWAT, while the effect was not or poorly dependent on iBAT activation [7]. In the present experiment also HLS, the rate-limiting enzyme for acylglycerol hydrolysis in adipocytes [15] was upregulated by TLQP-21, although the effect was significant only versus SD fed mice. It is known that the molecular machinery regulating energy expenditure and lipolysis in the adipose organ is under direct control of sympathetic nerves abundantly entering into this tissue, norepinephrine released from the sympathetic nerve endings stimulates mostly the β3-AR and activates HSL and UCP1 [20, 59]. Our results suggest that increased β-AR-mediated signaling may induce downstream events including activation of HSL [27] and associated lipolysis. In addition, PPAR-δ regulates fatty acid transport and oxidation and stimulates thermogenesis in adipose tissue [5, 38], which is compatible with the effects observed. Reportedly, the whole signaling pathway except for UCP1 is upregulated following TLQP-21 treatment. UCP1 was clearly upregulated in the WAT following central TLQP-21 in mice fed a standard diet [7] suggesting a transdifferentiation of white into brown adipocytes [18]. In the present study we could not replicate this finding. UCP1 has been found to be unchanged, upregulated or downregulated in the epididymal eWAT during high fat diet [49, 54, 68]. It is therefore

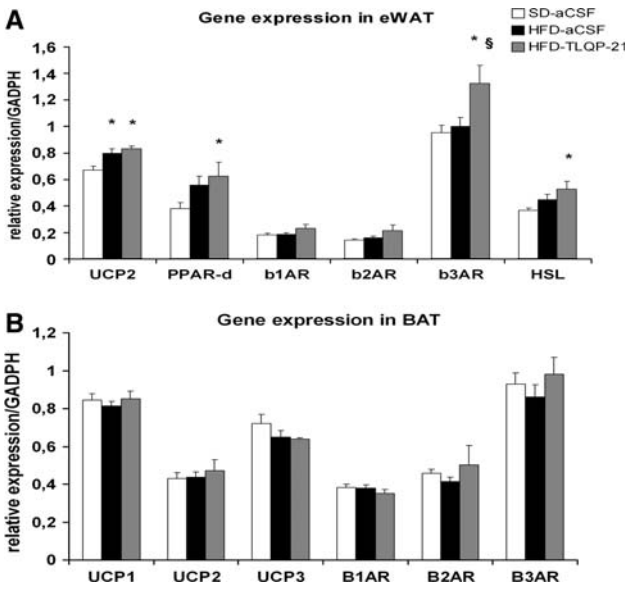

Fig. 4 Gene expression in adipose tissue. The figure shows gene expression (normalized over GADPH expression) in the epididymal white adipose tissue (**a**) and the interscapular brown adipose tissue (**b**) in mice fed a standard (SD) or a high fat diet (HFD) and chronically intracerebroventricularly injected with artificial cerebrospinal fluid (aCSF) or TLQP-21. * and $^{\S}P < 0.05$ versus SD-aCSF and versus HFD-aCSF, respectively

premature to draw a definitive conclusion on its modulation and it is thus possible to tentatively conclude that in our study an interaction occurred between two different stimuli on UCP1 expression: HFD having a negative or neutral effect and TLQP-21 determining a stimulatory effect. On the contrary, without any effect of TLQP-21, the mitochondrial UCP2 was clearly upregulated by the high fat feeding [1] in agreement with its putative role in the control of ATP synthesis, regulation of fatty acid metabolism and control of reactive oxygen species production in metabolically active tissues [12, 51].

Changes of gene expression were paralleled by a decrease in serum NE, an increase of E and an increase in the E/NE ratio, thus suggesting an alteration in the normal catecholamines balance as it occurs in a number of adrenal pathological conditions [24]. It is known that denervation of adipose pads is associated with decreased NE and increased expression of β3-AR while the opposite occur with adrenalectomy [31, 53]. β3-AR are more stimulated by local NE and less by E than for example β2-AR and this is due to the reduced affinity to E of β3-AR compared to β1- and β2-AR [55]. However, E may stimulate β3-AR-induced adenylate-cyclase activity [4] and dose-dependently activate lipolysis in vivo [13]. Accordingly, the mechanism which we hypothesize for the effects of TLQP-21 on adipose tissue is the following: TLQP-21 acting centrally (mechanism still to be determined) would decrease sympathetic tone, resulting in overall decreased NE release in the eWAT nerve endings, thus determining an up-regulation of β3-AR (and to a lower extent also of β1-AR and β2-AR); additionally, TLQP-21 would determine increased E release from chromaffin cells in the adrenal medulla [of note, a recent study identified TLQP-immunostaining in E- but not in NE-chromaffin cells in the adrenal medulla of bovine, swine and rat [23]; the net result would be increased E-stimulated β-AR mediated catabolic effects in the fat pads and resulting resistance to obesity. In agreement, preliminary data from our group show that central TLQP-21 may limit immobilization-induced NE release while increasing late E release (Bartolomucci et al., unpublished observations). Alternative explanations would be that: (1) TLQP-21 may normalize the higher basal sympathetic activity described in DIO-prone rats [44, 45]; (2) chronic TLQP-21 infusion may modulate pre-existing differences in adipose tissue gene expression which can predispose mouse to be probe to obesity [39].

In conclusion, we have demonstrated that chronic icv infusion of TLQP-21 prevented diet-induced obesity despite overfeeding associated with the palatable high fat diet. Resistance to fattening was associated with: (1) overall increase of the expression of catabolic markers, the most significant being an increase in β3-AR, in the adipocyte and resulting normalization of hormonal changes usually associated with obesity, i.e., leptin increase and ghrelin decrease; (2) increased E/NE ratio which we speculate could the working mechanism of action of central TLQP-21 activity. Present results are particularly relevant because they: (1) have been obtained in a subpopulation of mice which increased body weight upon exposure to high fat diet; (2) occurred in presence of diet-induced hyperphagia; (3) replicated and extended our original findings with mice fed a standard diet and treated with TLQP-21; (4) further indicated that TLQP-21 may be an important novel target in anti-obesity drug programs [22, 40].

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