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Cyclic glycine-proline administration normalizes high-fat diet-induced synaptophysin expression in obese rats

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ABSTRACT

Childhood metabolic disorders are associated with insulin-like growth factor (IGF)-1 deficiency, which can adversely affect brain development and function. As a neuropeptide, cyclic glycine-proline (cGP) improves IGF-1 function in brain and regulates IGF-1 bioavailability in plasma. Whether such a regulatory process mediates the neurotrophic effects of cGP remains unknown. This study examined the effects cGP treatment on synaptic expression and their association with IGF-1, IGF binding protein (IGFBP)-2 and cGP concentrations in the brain of rats with high fat diet (HFD)-induced obesity.

Male rats received either a HFD or a standard chow diet (STD) from weaning and were then treated with either saline or cGP from 11 to 15 weeks of age. The concentrations of cGP, IGF-1 and IGFBP-2 were measured in the brain tissues using ELISA and HPLC-MS. The expressions of synaptic markers were evaluated in the hippocampus, hypothalamus and striatum using immunohistochemical staining.

Compared to the STD group, IGF-1 and IGFBP-2, but not cGP concentrations, were lower in the HFD groups. The expression of hippocampal synaptophysin, glutamate receptor-1, GFAP and striatal tyrosine-hydroxylase were also reduced in the HFD groups. While treatment did not alter tissue IGF-1, cGP administration that increased the concentration of cGP in brain tissues, normalized the expression of synaptophysin, GFAP and tyrosine-hydroxylase, but not glutamate receptor-1. IGF-1 concentration in brain tissues correlated with the expression of all synaptic markers.

HFD feeding reduced synaptic expression and tissue IGF-1 in brains which were closely associated, thus suggesting IGF-1 in the brain is largely bioavailable. Without increasing IGF-1 in the brain, administration of cGP normalized synaptic expression, possibly be mediated through increasing bioavailable IGF-1, but further studies are required to confirm this.

1. Introduction

Insulin-like growth factor-1 (IGF-1) function is critical for normal postnatal brain development and brain function in adulthood (O'Kusky et al., 2000; Dyer et al., 2016). A high-fat diet (HFD) during childhood can impact on the central IGF-1 axis (Bhat and Thirumangalakudi, 2013). IGF-1 function in brain is highly regulated through endocrine, paracrine and autocrine processes (Gluckman et al., 1992; Guan et al., 1996; Mohan and Baylink, 2002; Chesik et al., 2007; Fernandez et al., 2007). The majority of brain IGF-1 is transported from circulation where growth hormone controls liver production of IGF-1 (Fernandez et al., 2007). The local induction of IGF-1 in brain tissues is mainly from glial cells, which can be enhanced after brain injury (Gluckman et al., 1992). Similar to the autocrine regulation of circulating IGF-1, the

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reversible binding of IGF binding proteins determines the bioavailability of IGF-1 in brain tissues (Chesik et al., 2007).

Cyclic glycine-proline (cGP) is a small metabolite naturally cleaved from the N-terminal of IGF-1, cGP is neuroprotective and improves brain development and function following peripheral administration (Singh-Mallah et al., 2016a; Guan et al., 2014). It is also known that cGP regulates the bioavailability of circulating IGF-1 by competing with the binding of IGF-1 to insulin-like growth factor binding protein (IGFBP)-3 (Singh-Mallah et al., 2016a; Guan et al., 2014; Guan et al., 2018). This competitive binding is concentration dependent (Guan et al., 2014), thus lower IGFBP-3 and higher cGP increases bioavailable IGF-1 (Guan et al., 2018). Endogenous cGP in brain tissues (Gudasheva et al., 1996) is generated from the same enzyme that cleaves cGP from plasma IGF-1 (Yamamoto and Murphy, 1995). Characterized as a neuropepide, cGP prevents ischemic brain injury through normalizing the phosphorylation of IGF-1 receptors (Guan et al., 2014). Whether this is achieved by increasing IGF-1 concentration through endocrine or paracrine regulation or improving the amount of bioavailable IGF-1 in brain tissues remains unknown.

Childhood metabolic disorders can impair cognitive development with abnormal synaptic function (Nguyen et al., 2017; Morin et al., 2017; Edlow, 2017; Liu et al., 2014). Exposure to a HFD during the peak period of brain development can also alter neuroplasticity that links to eating disorders (Sestan-Pesa and Horvath, 2016). The hippocampus and striatum are the brain regions involved in cognitive function (Reichelt, 2016; Guillermo et al., 2015; Guan et al., 2010). Expression of synaptic markers in the hippocampus are broadly used to evaluate brain function in rodents (Liu et al., 2014; Guillermo et al., 2015; Guan et al., 2010). Synaptophysin that labels the synaptic vesicles is a common marker for non-specific presynaptic function. The expression of neurotransmitters and receptors are also common markers for representing specific synaptic trafficking (Guillermo et al., 2015; Guan et al., 2010) including glutamate receptor-1 (GluR-1 or AMPA receptor) (Keifer and Zheng, 2010) and terminal expression of dopamine (Reichelt, 2016). Astrocytic expression is also associated with the regulation of synaptic neurotransmission (Newman, 2003).

cGP is a small neuropeptide, crosses the blood–brain barrier (Fan et al., 2018) and is orally bioavailable. (Singh-Mallah et al., 2016a; Guan et al., 2014; Gudasheva et al., 1996). It is also a natural nutrient and is present in rat breast-milk and foods (e.g. blackcurrant extracts) (Singh-Mallah et al., 2016a; Fan et al., 2018). Maternal administration of cGP before weaning improves brain development and function in adolescent rats (Singh-Mallah et al., 2016a). These data suggest that cGP plays a role in post-natal brain development and cognitive function. The effects cGP in the brain have been shown to be mediated by improvements in IGF-1 function (Guan et al., 2014), possibly through endocrine, paracrine and/or autocrine regulation of IGF-1. The current study examined the effects of HFD and cGP treatment on the concentration of IGF-1, cGP and IGFBP-2 in brain tissues and their association with changes in synaptic expression in a rat model of HFD-induced metabolic disorders.

2. Materials and methods

2.1. Experimental procedures

A rat model of HFD-induced obesity was used as previously described (Segovia et al., 2017). All experimental procedures were approved by a local Animal Ethics Committee. Fig. 1 shows the experimental procedure and timeline. In brief, 48 male Sprague-Dawley weaners (21 days old) were housed under standard conditions (22 °C; 40–45% humidity 12 h light - 12 h dark daily photoperiod). Rats were randomly divided into two dietary groups: standard chow diet (STD; 3.1 kcal/g, 24% kcals from protein, 18% kcals from fat, 58% kcals from carbohydrate, Diet 2018, Harlan Teklad, Madison, WI; n = 24 per group) or HFD (4.7 kcal/g, 20% kcal from protein, 45% kcals from fat, 35% kcals from carbohydrate, D12451, Research Diets, NJ, USA; n = 24) for the duration of the experiment.

After 8 weeks of dietary exposure, each group was further divided into two treatment groups (n = 12 per group). Saline or cGP (3 mg/kg/day dissolved in saline) was delivered for 4 weeks through a subcutaneously implanted osmotic minipump (ALZET Osmotic pumps, Durect Corporation, Cupertino, CA, USA). The dose of cGP used is well-established for chronic treatment in rats (Singh-Mallah et al., 2016a; Guan et al., 2010). At the end of experiment, brains were perfused with ice-cold saline and then separated into two hemispheres. The right hemispheres were fixed with 4% paraformaldehyde in situ for at least 48 h before stored in 25% sucrose. The left hemispheres were frozen in liquid nitrogen and stored in – 80 °C until analysis.

2.2. Osmotic pump implantation

The procedure for the osmotic pump implantation has been described previously (Singh-Mallah et al., 2016a). Briefly, ALZET® osmotic pumps (2ml4, DURECT Corporation, CA, USA) were filled with either cGP/saline or saline only, then primed in normal saline at 37 °C for 48 h prior to implantation. The rats were anaesthetized using isoflurane (2–5% O_2 4 L/min). A small skin incision was made in the scapular region and the pump implanted subcutaneously. The wound was closed with sutures and an analgesic (paracetamol, 150 mg/kg/day, oral) was administered for 3 days following surgery.

2.3. Tissues extraction and analysis of IGF-1 and IGFBP-2

Frozen tissues from the left hemispheres were crushed into fine powder in liquid nitrogen. The tissue powder (200 mg) of each rat was mixed with ice-cold lysis buffer (0.5 ml, 50 mM Tris-HCl pH 8, 150 mM NaCl, 0.1% Triton X-100), diluted with the Halt protease and phosphatase inhibitor cocktail (1:100, Pierce Biotechnology) and homogenized using an electric tissue homogenizer (Omni TH® tissue homogenizer, TH-220, GA, USA). The samples were placed in ice and vortexed every 5 min for a total of 20 min, then centrifuged at 13,000 rpm for 20 min at 4 °C. The supernatants were then collected and transferred to a pre-washed Amicon Ultra 3 kDa centrifugal filter (Merck Millipore), then centrifuged at 14,000 ×*g* for 20 min at room temperature. The concentrate was collected from the filter device and total protein was measured using Direct Detect® Infrared Spectrometer (Merck, Germany). All samples was equalized to a total protein concentration of 26 mg/ml and were assayed with rat-specific ELISA in accordance with the manufacturer's instructions (Crystal Chem Inc., IL, USA).

2.4. Tissue extraction of cGP

The method was modified based on previous publications (Singh-Mallah et al., 2016a; Gudasheva et al., 1996; Fan et al., 2018). Tissue powder (300 mg) from each rat was homogenized in ice-cold water (0.5 ml) using an electric homogenizer (Omni TH® tissue homogenizer, TH-220, GA, USA). The homogenates were placed in ice for 20 min and vortexed every 5 min, then centrifuged at 13,000 rpm for 20 min at 4 °C. The homogenization procedure were repeated four times and the pooled supernatant dried using a vacuum concentrator (Savant SC250EXP, Thermo Scientific, Asheville, NC, USA) at 0.5 mTor for 4 h at room temperature. The dried powder was reconstituted in 0.5 ml of water and remaining residues were cleared by centrifuging at 13,000 rpm for 5 min. Protein concentrations were determined using the Direct Detect® Infrared Spectrometer (Merck, Germany)and equalized to a total volume of 400 µl with a final protein concentration of 10 mg/ml. Standards were prepared by reconstituting commercially synthesized cGP (Bachem, Switzerland) in water with a concentration range from 0.072 to 278 ng/ml. Two samples of known cGP concentrations were used as positive controls for the assay. All standards

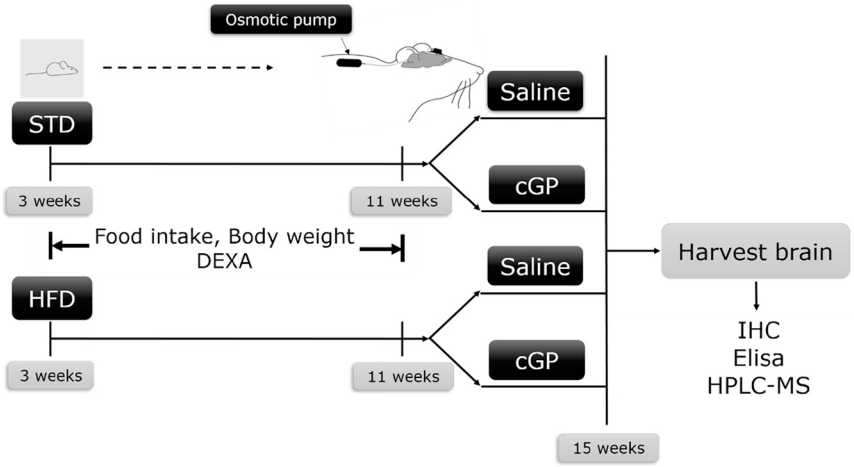

Fig. 1. Experimental timeline and procedures.

and testing samples received 50 µl of 200 ng/ml cGP-1,5,6,7,8-^{13}C, 4-^{15}N (cGP-5x^{13}C,1x^{15}N), an internal standard for the assay. The solution was vortex mixed, transferred to the pre-washed Amicon Ultra 3 kDa centrifugal filter and centrifuged at 14,000 × *g* for 30 min at room temperature. The filtrate was dried using a vacuum concentrator at 0.5 mTorr for 3 h at room temperature, then reconstituted in 80 µl of 5% methanol in water (v/v). The solution was transferred to an ultra-pressure liquid chromatography (UPLC) vial and centrifuged at 500 rpm at 4 °C to precipitate any remaining residues.

2.5. High performance liquid chromatograph-mass spectrometry (HPLC-MS) for cGP assay

The method of HPLC-MS for cGP analysis has been previously described (Singh-Mallah et al., 2016a; Fan et al., 2018). Briefly, the chro matography conditions consisted of a Synergy Hydro 2.5 µm column 100 × 2 mm (Phenomenex) with an initial mobile phase composition of 10% methanol / 90% water flowing at 200 µl per minute with a column temperature of 35 °C. Ionization was undertaken using heated electrospray ionization in positive mode with a voltage of 4000 V, a sheath gas flow of 30 psi, an auxiliary gas flow of 2 psi, and a capillary temperature of 250 °C. Fragmentation was achieved with argon at 1.2 mTorr as the collision gas and a dissociation voltage of 35 V. The mass spectrometer system consisted of an Accela MS pump and autosampler on a Finnigan TSQ Quantum Ultra AM triple quadrupole mass spectrometer all controlled by Finnigan Xcalibur software (Thermo Electron Corporation, San Jose, CA, USA) The mass spec was run in selective reaction monitoring mode with the following two transitions 155.1 - > 70.2 *m/z* and 157.1 - > 70.2 *m/z* utilised for cGP and cGP-5x^{13}C,1x^{15}N respectively. The retention time for both peaks was 3.6 min. Unknown samples were quantified using the peak area ratio of cGP/cGP-5x^{13}C,1x^{15}N compared with the standard curve of known concentrations.

2.6. Immuno-histochemical staining

Brain tissue preparation and immuno-histochemical staining techniques have been described previously (Guillermo et al., 2015; Guan et al., 2010). Sequential sections (50 µm, with intervals of 24 sections e.g.1, 25, 49, 73, ….) that contain either the hippocampus/hypothalamus or the striatum were sectioned. The sections were pre-treated with 1% H_2O_2 and 50% MeOH in 0.2% PBS-Triton-X for 30 min, then with 1.5% normal sheep serum/phosphate-buffered saline for 1 h at room temperature. The sections were then incubated with the following primary antibodies: mouse anti-glial fibrillary acidic protein (GFAP, Sigma, 1:5000) for astrocytes, mouse anti-synaptophysin (Sigma, 1:10,000) for synaptic vesicles, rabbit anti- glutamate receptor-1 (GluR-1, Chemicon, 1:5000) for glutamate receptor-1 or rabbit anti-tyrosine hydroxylase (TH, Protos Biotec Corp, 1:2000) for dopamine terminal straining at 4 °C for 48 h respectively. The sections were then incubated either with biotinylated horse anti-mouse or goat anti-rabbit secondary antibodies accordingly (Sigma, 1:2000) at 4 °C overnight. Finally, the brain sections were incubated with Extra Avidin-HRP (Sigma 1:2000) for 2 h at room temperature. Staining was visualized using 0.05% DAB (w/v) with 0.01% H_2O_2 in 0.1 M phosphate buffer for 15 min. The sections were washed 3 times using the PBST (PBS with 0.2% Triton X-100) between each step of the procedure. Sections were mounted on glass slides, air-dried, dehydrated through a graded alcohol series and cover-slips were applied.

2.7. Data assessment

The assessment and analysis of immunostaining have been described previously (Guillermo et al., 2015; Guan et al., 2010). In brief, at least 4 sequential sections from each rat brain were used for assessing each staining parameter. Images were acquired by light microscopy (Nikon 800, Tokyo, Japan), imaged using SigmaScan (Pro 5.0, SPSS, Chicago, USA) and analyzed using Image J (Image J Version 1.50i, Wayne Rasband, NIH, USA). The average density of synaptophysin was analyzed in the CA1-2, CA-3 (mossy-fiber) and CA-4 sub-regions of the hippocampus. The average densities of the TH and GluR-1 were analyzed in the striatum and hippocampus as one region respectively. The percentage of area with GFAP staining was assessed in the CA-4 sub-region of the hippocampus (Guillermo et al., 2015). The value derived from all sections of each rat was averaged and used for statistical analysis.

2.8. Statistical analysis

Data were analyzed using GraphPad Prism 7 (version 7.03, ©1992–2016 GraphPad Software, Inc.). Two-way ANOVA was used for analyzing the main effects of treatment, diet and the interactions between diet and treatment. Tukey's multiple comparison were used for identifying specific effects of diet and treatment. Pearson tests were used for analyzing correlations. Data are presented as mean ± SEM with the significance level set at $p < .05$.

3. Results

3.1. The effects of diet and treatment on IGF-1, IGFBP-2 and cGP in brain tissues

Compared to the STD group there was an overall decrease in IGF-1

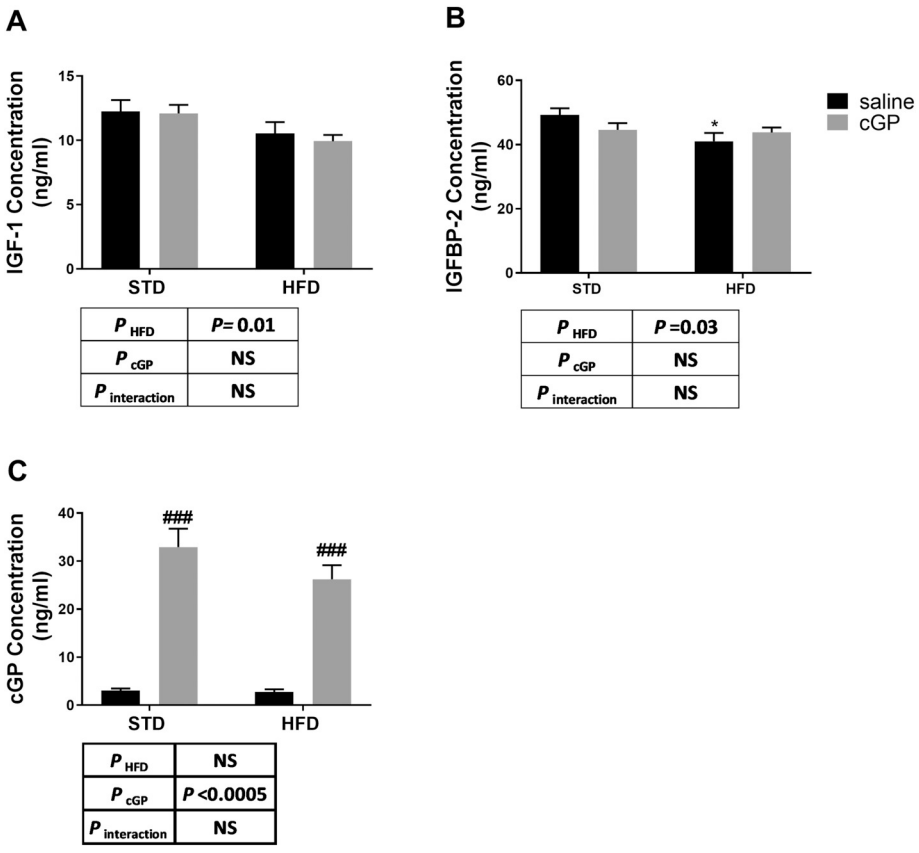

Fig. 2. Effects of HFD and cGP treatment on the concentrations of IGF-1 (A), IGFBP-2 (B) and cGP (C) in brain tissues. The main effects are presented in the inserted tables and specific effects presented within the figures. *p < .05 indicates dietary effect; ###p < .0001 indicates cGP treatment effect. Data are presented as mean ± SEM, n = 11–12 per group.

(p = .012, f (1,44) = 6.73, Fig. 2A) and IGFBP-2 (p = .036, f (1, 44) = 4.63, Fig. 2B) concentration in the brain tissues in the HFD group. Multiple comparison analysis showed a lower IGFBP-2 concentration in HFD/saline group compared to STD/saline group and cGP treatment did not alter IGF-1 or IGFBP-2 concentrations. The dietary effects on IGF-1 and IGFBP-2 were independent of treatment. Treatment with cGP increased the brain concentration of cGP (p < .0001, f (1, 43) = 102.6, Fig. 2C) in both STD (p < .0001) and HFD groups (p < .0001). There was no HFD effect on cGP concentration and the treatment effects of cGP were independent of the diets.

3.2. Changes of synaptophysin in the hypothalamus and the sub-regions of the hippocampus

Representative images of immunohistochemical staining of synaptophysin in the hypothalamus (Fig. 3A) and hippocampus (Fig. 3B) show the area used to analyse the expression. Synaptophysin staining was strongly expressed in the mossy-fiber area of the CA-3 and its extension into the CA-4 sub-region. The staining in the CA1-2 and hypothalamus regions were relatively less intensive.

The average density of synaptophysin in the hypothalamus was similar between diet and treatment groups. However, there was a significant interaction between diet and treatment (p = .0007, f [1, 20] = 15.9, Fig. 3C). Compared to the HFD/saline group, cGP treatment significant reduced synaptophysin density in the HFD/cGP group (p = .0094). There was a main treatment effect of increasing the expression in the CA 1-2 sub-region of the hippocampus (p = .025, f [1, 24] = 5.71, Fig. 3D) with a significant interaction between diet and treatment (p = .04, f [1, 24] = 4.36). Compared to the STD/saline group, the average density of synaptophysin was reduced in the CA1-2 sub-region in the HFD/saline group (p = .041, Fig. 3D). Compared to HFD/saline group, synaptophysin density was increased in the CA1-2 sub-region of the HFD/cGP group (p = .03). There was neither a diet nor a treatment effect on the mossy-fiber area of the CA-3 sub-region, with a significant interaction between treatment and diet (p = .004, f [1, 21] = 10.16, Fig. 3E). Compared to the STD/saline group, the synaptophysin density in the mossy-fiber region was reduced in the HFD/saline group (p = .03). Compared to the HFD/saline group, cGP treatment increased the density in the mossy-fiber area in the HFD/cGP group (p = .02, Fig. 3E). There was no diet or treatment effect on the CA-4 sub-region of the hippocampus, but a significant interaction was observed between diet and treatment (p = .0041, f = [1, 25] = 4.641, Fig. 3F). Compared to the HFD/saline group, cGP treatment significantly increased the density in the CA-4 sub-region of HFD/cGP group (p = .049).

3.3. Changes of TH in the striatum

The expression of TH, which stained the terminals of projecting dopamine neurons and represented dopamine trafficking at pre-synapses, was specifically distributed within the striatum (Fig. 4A).There was a main diet effect on reducing TH expression (p = .008, f [1, 24] = 2.87, Fig. 4B), but no treatment effect and no interaction between the diet and treatment. Compared to the STD/saline group, the average density of TH was reduced in the HFD/saline group (p = .011, Fig. 4B). There was no statistical differences between the HFD/saline and HFD/cGP groups.

3.4. Changes of GFAP in the CA-4 sub-region of the hippocampus

There was no obvious morphological changes in astrocytes across the groups (Fig. 5A). The strongest expression of GFAP positive astrocytes was in the CA-4 sub-region where the GFAP expression was assessed (Fig. 5B). There were main diet effect on reducing GFAP expression (p = .044, f [1, 22] = 4.546, Fig. 5C) and treatment effect on increasing (p = .032, f [1, 22] = 5.21, Fig. 5C) the percentage of area

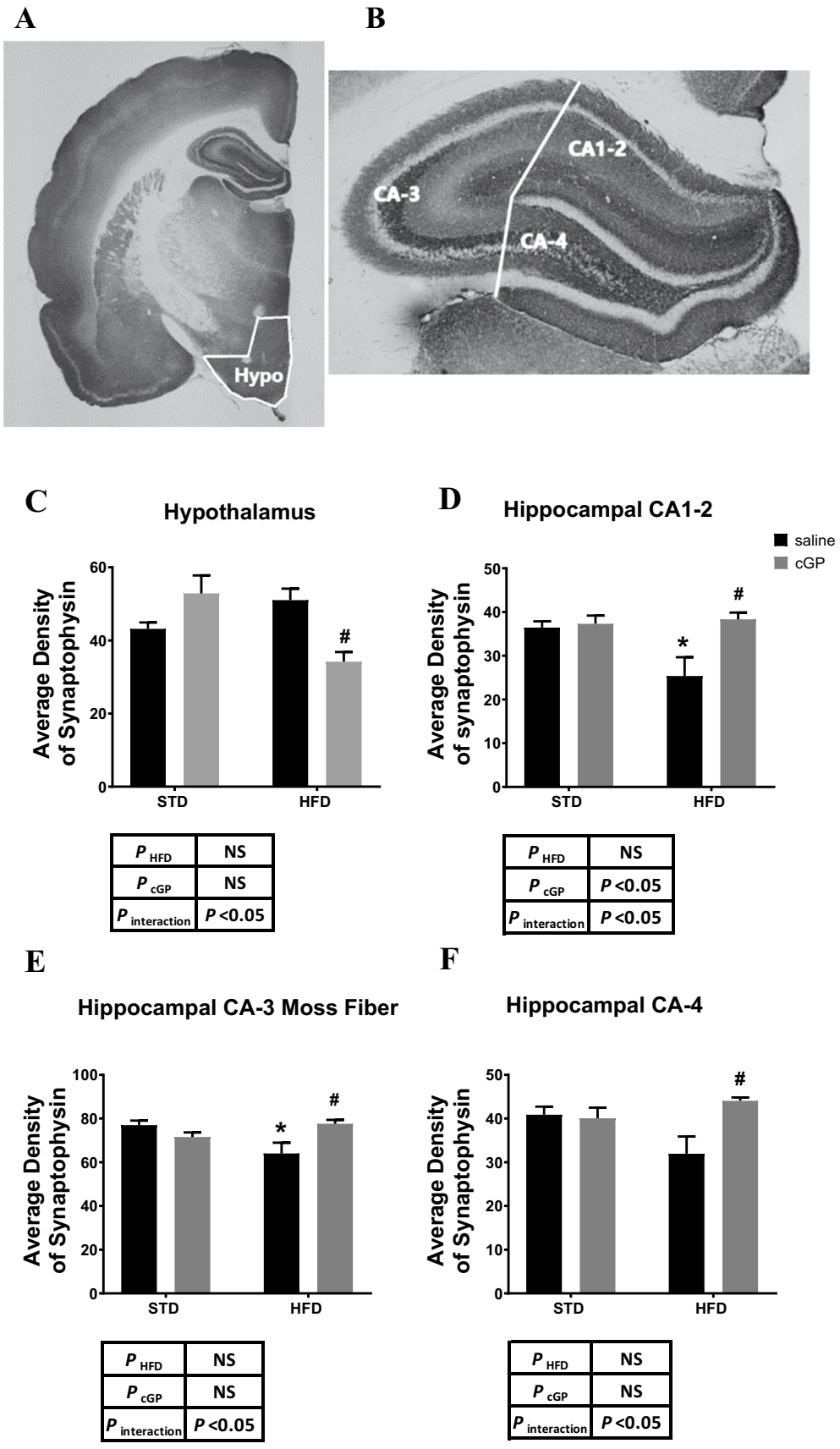

Fig. 3. Microscopy images show the distribution and area used for assessing synaptophysin in the hypothalamus (A) and the CA1-2, CA-3 (mossy fiber) and CA-4 sub-regions of the hippocampus (B). The diet and treatment effects on average density of synaptophysin in the hypothalamus (C), the CA1-2 (D), CA-3 (mossy fiber area, E) and CA-4 of the hippocampus (F) were presented. The main effects presented in the inserted table and specific treatment and diet effects presented in the figure. *p < .05 indicate HFD effects; #p < .05 indicates cGP treatment effects. Data are presented as mean ± SEM, n = 5–8 per group, Scale bare = 200 μm.

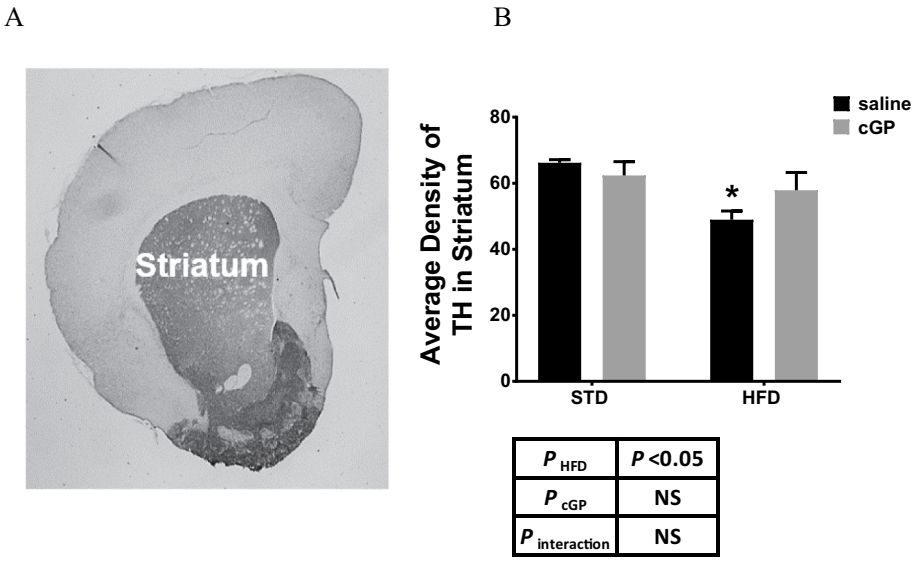

Fig. 4. The expression of TH was assessed in entire striatum regions where TH staining was evenly distributed (A). The diet and treatment effects on average density of TH staining in the striatum were presented (B). The main effects presented in the inserted table and specific treatment and diet effects presented in the figure. *p < .05 indicate HFD effects. Data are presented as mean ± SEM, n = 6–8 per group.

staining of GFAP with no interaction between diet and treatment. Compared to the HFD/saline group, there was a trend toward an increase in the percentage of area staining in HFD/cGP group (p = .057). A positive correlation was found between the expression of GFAP and the average density of synaptophysin in the CA-4 (p < .0001, r = 0.643, Fig. 5D).

3.5. Changes of GluR-1 in the hippocampus

The distribution of GluR-1 expression was relatively lighter in the

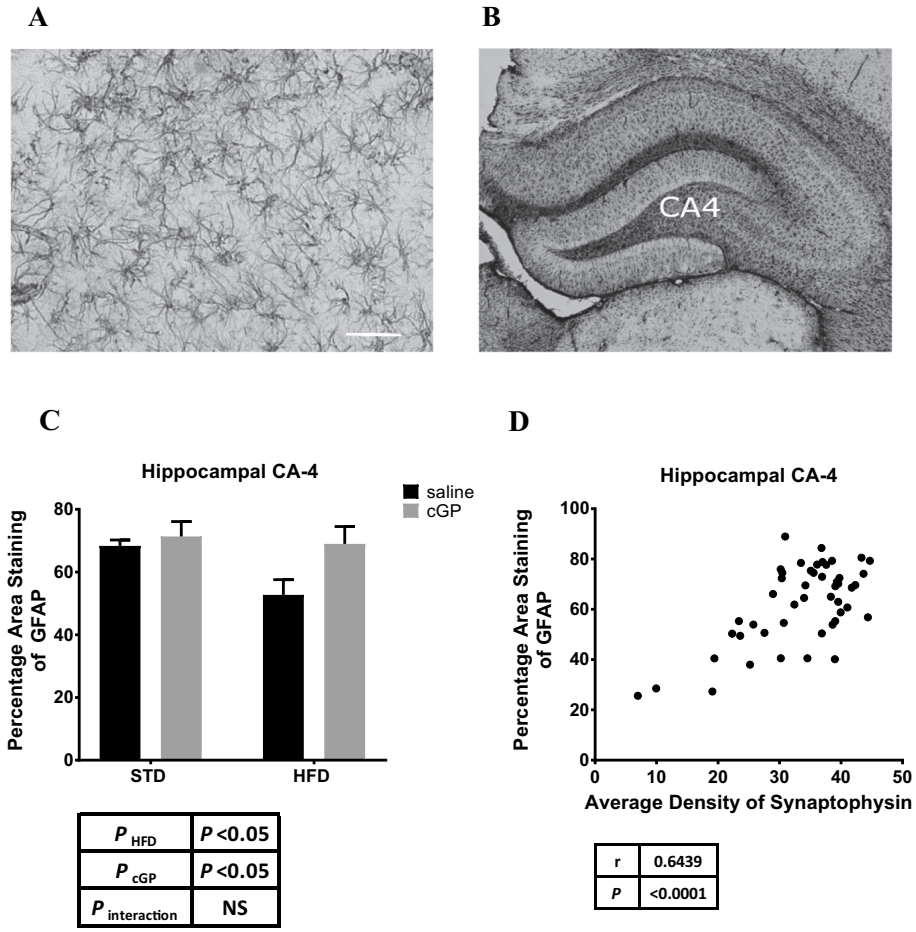

Fig. 5. Shows the morphological changes of GFAP positive astrocytes in the CA-4 sub-regions of the hippocampus (A) and the area used for accessing the expression of GFAP staining (B). The main effects of diet and treatment were presented in the inserted table (C). The area expression of GFAP was significantly correlated with the density of synaptophysin in the CA-4 sub-region of the hippocampus (D). Data are presented as mean ± SEM, n = 6–8 per group, Scale bare = 200 μm.

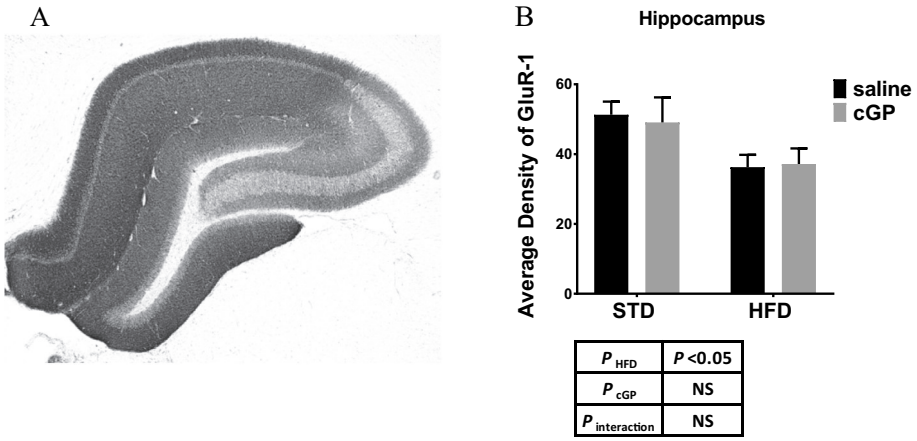

Fig. 6. The microscopy photo image shows the expression of GluR-1 in the hippocampus (A) and the bar graph shows the effects of diets and treatment on GluR-1 expression (B). The main effects presented in the inserted table. Data are presented as mean ± SEM, n = 6–8 per group.

mossy fiber areas of CA-3 and CA-4 sub-regions of the hippocampus (Fig. 6A). There was a main diet effect on GluR-1 ($p = .012$, f [1, 24] = 7.258) with no treatment effect and no interaction between diet and treatment (Fig. 6B).

3.6. Correlation of synaptic markers with brain IGF-1 concentration

The concentrations of IGF-1 in the brain tissues were significantly correlated with the expression of the synaptophysin in the CA1-2 ($r = 0.55$, $p = .0018$, Fig. 7A), CA-3 ($r = 0.48$, $p = .015$, Fig. 7B) and CA-4 ($r = 0.52$, $p = .0027$, Fig. 7C) sub-regions of the hippocampus; the TH in the striatum ($r = 0.67$, $p < 0.0001$, Fig. 7D); the GFAP in the CA-4 sub-region of the hippocampus ($r = 0.53$, $p = .0053$, Fig. 7E) and GluR-1 in the hippocampus ($r = 0.73$, $p < 0.0001$, Fig. 7F). The changes in IGFBP-2 and cGP were only correlated to the expression of TH in the striatum ($r = 0.44$, $p < .014$) and the synaptophysin in the CA1-2 ($r = 0.45$, $p < .014$) respectively (Supplemental Table 1).

4. Discussion

In general, a post-weaning HFD reduced IGF-1 concentration and the expression of synaptic markers in the brain of young adult rats. The changes in IGF-1, but not IGFBP-2 and cGP, concentration were associated with the expression of synaptic markers. Thus the endogenous brain IGF-1 appeared to be largely bioavailable. Administration of cGP that increased brain cGP, but not IGF-1 and IGFBP-2, had normalized the HFD-induced changes in synaptic expression. If further confirmed, the effects of cGP may be mediated through further improving bioavailability of IGF-1 rather than increasing IGF-1 in the brain tissues.

The IGF-1 concentration in brain tissues was reduced in HFD groups, suggesting IGF-1 deficiency is associated with HFD-induced childhood obesity. IGF-1 play a critical role in post-natal brain development and function, thus IGF-1 deficiency may be a mechanism underlying reduced synaptic expression.

A truncated form of IGF-1, des-N-(1-3) IGF-1 (des-IGF-1) has been identified as a tissue form of IGF-1 in the brain (Sara et al., 1993). The truncation of IGF-1 reduces its binding affinity with IGFBPs, thus des-IGF-1 is more bioavailable than the full length IGF-1 (Sara et al., 1993). HFD reduced the concentration of IGF-1 in brain tissues and expressions of synapophysin, GluR-1 and GFAP in the hippocampus and TH in the striatum. Consistent with recent observations from others (Bhat and Thirumangalakudi, 2013; Reichelt, 2016), our data also suggested an adverse effect of HFD on synaptic function. The changes in IGF-1 concentration were closely associated with the level of synaptic expression, independent of treatment. The data thus support the role for IGF-1 in synaptic function (Dyer et al., 2016). With currently available assays unable to specifically detect des-IGF-1, the close association between the changes in brain IGF-1 and synaptic expression may suggest that brain IGF-1 is more bioavailable than plasma IGF-1, which is largely not bioavailable due to higher affinity binding of IGFBPs (Clark et al., 1993).

The truncation of IGF-1 in plasma also forms cGP. As the major binding part of IGF-1, cGP retains the binding affinity to IGFBP-3 (Guan et al., 2014). The competitive binding between cGP and IGF-1 to IGFBP-3 regulates the bioavailability of IGF-1 in circulation, in which lower IGFBP-3 and higher cGP increases the bioavailability of IGF-1 in plasma (Singh-Mallah et al., 2016a; Guan et al., 2014; Guan et al., 2018; Fan et al., 2018). Such autocrine regulation may not play a major role in regulating the bioavailability of IGF-1 in brain. A HFD also moderately reduced IGFBP-2 and did not alter cGP concentration in brain tissues. Unlike IGF-1, neither IGFBP-2 nor cGP concentrations were associated with synaptic changes in the brain (Fig. 7, Supplemental Table 1). IGFBP-2 is a main IGFBP in brain and has been shown to regulate the bioavailability of IGF-1 (Chesik et al., 2007; Russo et al., 2005). Whether this reduced IGFBP-2 represents an autocrine response in order to improve IGF-1 availability in the brain needs further investigation (Oh et al., 1993). The 'no change' of cGP may be an insufficient autocrine response of endogenous cGP that failed to maintain synaptic activity.

Administration of cGP increased its concentration in brain tissues and selectively normalized the expression of some synaptic markers and astrocytes in the HFD group. The efficacy of cGP is mediated through altered the regulation of IGF-1 function (Guan et al., 2014; Singh-Mallah et al., 2016b). For example the neuroprotective effects of cGP have been reported to be mediated through improving phosphorylation of the IGF-1 receptor (Guan et al., 2014). IGF-1 in the brain can be locally produced or transported from the peripheral circulation (Russo et al., 2005). Given that the treatment of cGP did not increase IGF-1 in brain tissues, whether the effects of cGP on synaptic expression are mediated through further improving the bioavailability IGF-1 in brain tissues (Graphical abstract), need further evaluation. The role of cGP in brain development and cognitive function has been documented (Singh-Mallah et al., 2016a; Gudasheva et al., 1996; Guan et al., 2010). Maternal administration of cGP during postnatal brain development improves learning and memory in adolescent rats (Singh-Mallah et al., 2016a) and treatment of cGP analogue improves cognitive function in human Rett syndrome (Glaze et al., 2017). Gudasheva et al. also demonstrated the effect of cGP on improving the performance of passive avoidance tasks in adult rats (Gudasheva et al., 1996). Treatment with a cGP analogue prevents scopolamine-induced memory impairment by normalizing expression of synaptophysin in the hippocampus, but also did not normalize the loss of GluR-1 expression (Guan et al., 2010). Effects of HFD on astrocytic response have been varied across studies

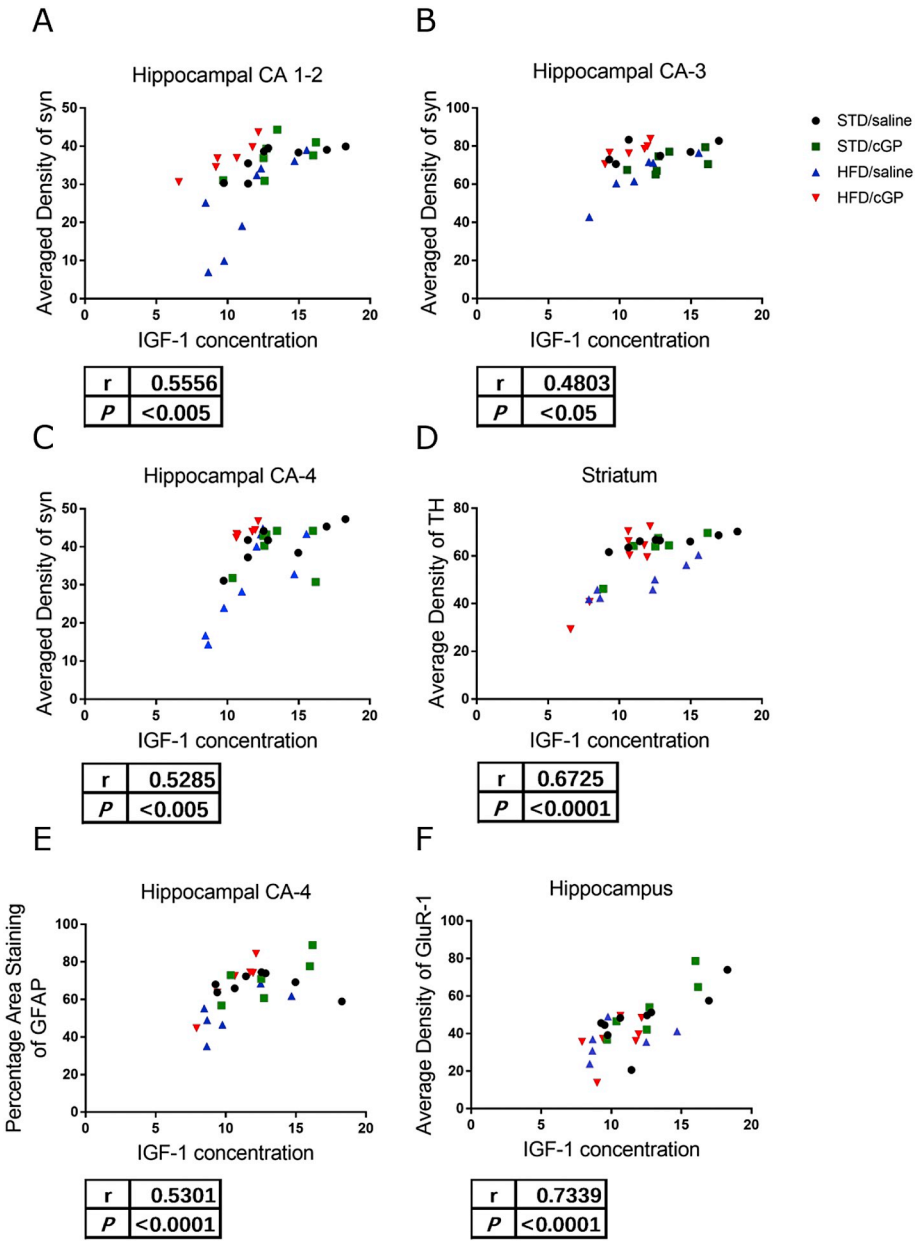

Fig. 7. Shows the correlations between IGF-1 and synaptic markers of each experimental group (color coded). The changes in IGF-1 were significantly correlated with the density of synaptophysin in the CA1-2 (A), CA-3 (B) and CA-4 (C) sub-regions of the hippocampus; the density of TH in the striatum (D); the area of GFAP staining in the CA-4 (E) and the density of GluR-1 in the hippocampus (F), n = 25–30.

(Balland and Cowley, 2017). Apart from regulating inflammation and vascular remodeling, astrocytes regulate the dynamics of synaptic neurotransmission at pre-synaptic terminals (Newman, 2003), particularly in the hippocampus (Rouach et al., 2008), where we found a close association with synaptophysin. The reduced GFAP expression is unlikely to be an inflammatory response to the HFD, thus the treatment effects of cGP on GFAP expression may provide further support for a role for cGP in synaptic function.

The hypothalamus mediates energy metabolism and appeared to respond HFD-induced metabolic disorders differently (Jennings et al., 2013). Even though the HFD effect on synaptophysin expression were not conclusive in the hypothalamus, cGP treatment significantly reduced the synaptophysin expression in the HFD group with an opposite trend toward an increase in STD group. The mode of cGP action is mediated through normalizing IGF-1 effects, which acts in either a promoting or inhibitory manner (Guan et al., 2014; Guan et al., 2010; Singh-Mallah et al., 2016b). Thus cGP shares the same mechanism of IGF-1 in developmental brain function (O'Kusky et al., 2000). The lack of cognitive function testing is a limitation of the current study. However, the dietary impact on synaptic changes may or may not translate to behavioral outcomes (Nguyen et al., 2017) during the early stages of pathophysiological changes.

5. Conclusion

Unlike the limited bioavailability of IGF-1 in plasma, brain IGF-1 appeared to be largely bioavailable, possibly due to its truncation of cGP. The reduced tissue IGF-1 may represent a HFD-induced impairment in synaptic function. Administration of cGP improved synaptic expression possibly be mediated through autocrine regulation that improved the bioavailability of IGF-1 rather than increasing tissue IGF-1 in brain tissues.

Author contributions

FL, conducted histological and majority biological assays and analysis, statistical analysis, data presentation and assisted on writing the manuscript; KL, conducted experimental tests and biological assays, developed assays; AW, conducted biological assays; MV, provided inputs on experimental design and revised manuscript; PH designed and syntheses international standard for cGP assays and edited manuscript; JG generated the hypothesis, provided the funding, designed experiments and wrote manuscript.

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Declaration of Competing Interest

The authors have no conflict interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.npep.2019.05.006.

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