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Research article

Long term Westernized diet leads to region-specific changes in brain signaling mechanisms

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ABSTRACT

Western diets, high in fat and energy, are associated with cognitive deficits in humans and animal models, but the underlying mechanisms are not fully elucidated. This includes whether diet-induced dyslipidemia *per se* negatively impacts brain signaling. Here we investigate the effects of dyslipidemia induced by two high fat diets with or without high sucrose on hippocampal and frontal cortical oxidative stress, brain-derived neurotrophic factor (BDNF) and down-stream markers of synaptic plasticity, as well as alterations in monoaminergic neurotransmitter levels. A high fat diet was associated with decreased antioxidant status (vitamin C), increased serotonin in the frontal cortex, and increased ratio of phosphorylated Ca^{2+}/calmodulin-dependent protein kinase II in the hippocampus, while a high fat and sucrose diet decreased levels of vitamin C in the frontal cortex and BDNF in the hippocampus. Markers of dyslipidemia correlated significantly with cerebral vitamin C levels, monoaminergic neurotransmitters and metabolites in the frontal cortex, but not in the hippocampus. Thus, a high fat diet caused regional alterations in antioxidant levels, neurochemistry and molecular markers in the non-obese dyslipidemic guinea pig.

1. Introduction

A Western diet characterized by a high fat/high caloric intake is associated with dyslipidemia and obesity, but also with cognitive deficits such as psychiatric diseases and dementia in humans [20,37,53], linking the current global pandemic of diet-imposed dyslipidemia and obesity to negative consequences in the brain [10,38].

Reports from studies of animal models exposed to long term diets, high in fat and/or sugar show reduced hippocampal memory functions [33,46,52], increased anxiety and depression-like behavior [23–25], as well as alterations of markers associated with Alzheimer's disease [18,48]. Several mechanisms have been implicated in dyslipidemia-induced cognitive impairments, including a decrease in the important growth factor, brain-derived neurotrophic factor (BDNF), altered synaptic plasticity, as well as increased oxidative stress [8]. Particular interest has been given to the effects of Western diets on frontal cortex- and hippocampus-dependent functions due to their involvement in memory, emotions, decision making and spatial navigation [5,44].

Increased oxidative stress has been shown to reduce BDNF [21,52]. BDNF is an important growth factor in the development of brain circuitry and function and is involved in dendrite and synapse development, memory formation, synaptic plasticity and neuronal survival [7,9]. Thus, alterations of BDNF levels may in turn impact plasticity markers such as synapsin 1 – involved in neurotransmitter release, synapse formation and maturation [6] – and Ca^{2+}/calmodulin-dependent protein kinase II (CAMKII) – important in long-term potentiation, synaptic plasticity and learning [17,41] – either through changes in expression levels or the degree of phosphorylation [4,33]. Furthermore, a decreased availability of vitamin C (vitC) may not only promote oxidative stress, but also directly impact synthesis and metabolism of monoaminergic neurotransmitters [27,51].

Hence, diet-induced dyslipidemia can be speculated to reduce neuronal signaling leading to reduced cognitive function. However, results are conflicting as some studies report no or even positive effects on overall brain function during a Westernized dietary regime [26,31], whereas other investigations report that cognitive deficits precede

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; APP, Amyloid Precursor Protein; BDNF, brain-derived neurotrophic factor; CAMKII, Ca^{2+}/calmodulin-dependent protein kinase II; CTRL, Control diet; DHA, dehydroascorbate; DOPAC, 3,4-dihydroxyphenylacetic acid; GFAP, Glial fibrillary acidic protein; HF, high fat diet; HFHS, high fat, high sucrose diet; HVA, homovanillic acid; NeuN, Neuronal nuclei protein; PSEN1, presenilin 1; p-CAMKII, phosphorylated Ca^{2+}/calmodulin-dependent protein kinase II; p-synapsin 1, phosphorylated synapsin 1; VitC, vitamin C

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obesity [22,35], suggesting that the state of dyslipidemia rather than obesity *per se* may be the driving force of a cognitive decline. Consequently, it is currently difficult to assess any isolated effects of either dyslipidemia or obesity.

Interestingly, the lipoprotein profile and markers associated with Alzheimer's disease are similar between guinea pigs and humans following a high fat/high cholesterol diet [3,12,43]. This study investigates the effects of long term, high fat diet-induced dyslipidemia in a non-obese guinea pig model on brain oxidative stress and potential regional alterations of BDNF and related markers of synaptic plasticity, as well as changes in levels of monoaminergic neurotransmitters. A potentially exacerbated effect by increased dietary sucrose in a high fat diet was also explored.

2. Materials and methods

2.1. Animals

All experiments were approved by the Danish Animal Experiments Inspectorate under the Ministry of Food, Agriculture and Fisheries and in accordance with EU directive 2010/63/EU, as previously described [19]. Tissue samples were utilized from a subset of animals included in a larger *in vivo* study, of which some data has previously been published [19]. In brief, female Hartley guinea pigs, 10 weeks of age (Charles River Laboratories, Kissleg, Germany), were weight-stratified into dietary groups receiving either *ad libitum* chow (CTRL, 4% fat, 0% sucrose, 0% cholesterol), high fat (HF, 20% fat, 0% sucrose, 0.35% cholesterol) or high fat, high sucrose (HFHS, 20% fat, 15% sucrose, 0.35% cholesterol) diet and group housed in floor pens. A detailed diet composition is provided in Table 1. Hay and water was provided *ad libitum* and animals were inspected daily by trained personnel. The animals were weighed once a week. No animals displayed signs of reduced welfare throughout the study period. After 25 weeks on the diets, the animals (n = 7 per group) were pre-anaesthetized with 0.08 ml/kg body-weight Zoletil-mix; Zoletil 50 (Virbac, Carros, France) supplemented with 0.75 ml Torbugesic vet (10 mg/ml, Scanvet, Fredensborg, Denmark) and 10 ml Rompun (10 mg/ml, Bayer, Leverkusen, Germany) diluted 1:10 in isotonic saline water and then placed on isoflurane (Isoba Vet 100%, Intervet International, Boxmeer, The Netherlands). After cessation of voluntary reflexes, intra-cardiac blood samples were obtained and the animals euthanized by decapitation, as previously described [19]. The brain was removed, rinsed in ice cold PBS, weighed, divided and snap frozen. The frontal cortex and the hippocampus were dissected on ice. Plasma markers of dyslipidemia have previously been published [19]. All animals appeared healthy and in very good condition with no signs of malnutrition or disease at inspection and at necropsy.

2.2. Antioxidant turnover

The levels of total vitC, dehydroascorbate (DHA) – the oxidized form of vitamin C – and resulting DHA% of total vitC in the frontal cortex were determined in tissue homogenate stabilized with equal amounts of *meta*-phos-phoric acid and as previously described [28]. Two animals were excluded for technical reasons.

2.3. Neurotransmitters

Monoaminergic neurotransmitters in the frontal cortex and the hippocampus were analyzed by HPLC as previously described [42].

2.4. Protein extraction

Forty mg tissue from each of the frontal cortex and the hippocampus were excised and subsequently homogenized in 500 µl cold RIPA buffer (50 mmol/l tris pH 8.0, 150 mmol/l sodium chloride, 1% Triton X-100, 0.5% sodium deoxycholate and 0.1% sodium dodecyl sulfate) with 1:100 protease inhibitor cocktail and 1:100 phosphatase inhibitor cocktail (Sigma-Aldrich, Darmstadt, Germany). The samples were centrifuged for 10 min at 12,000 rpm at 4 °C, the supernatant divided into aliquots and stored at −80 °C. To determine protein concentration, a commercially available BCA kit was used (Merck Millipore, Darmstadt, Germany) [47].

Table 1
Exact dietary composition of the experimental diets.
Reproduced from Ipsen et al. [19].

Nutrients (g/kg diet)	CTRL	HF	HFHS
Alfalfa	220	220	220
Wheat	283	290	103
Barley	180	–	–
Sucrose	–	–	150
Cellulose (lignocellulose)	46.0	40.0	46.0
Sunflower meal	30.0	30.0	30.0
Soybean meal	120	70.0	60.0
Soybeans (full fat)	26.0	26.0	26.0
Soybean concentrate	20.0	80.0	120
Soybean isolate (90% protein)	–	–	–
Amino acids	5.00	5.90	5.00
Vitamins & trace element[a]	10.0	10.0	10.0
Vitamin C (Stay-C)	29.0	29.0	29.0
NaCl	4.0	4.0	4.0
Calcium phosphate (monobasic)	12.9	15.4	16.4
Calcium propionate	5.5	5.5	5.5
Calcium carbonate	2.5	1.5	1.5
Choline Cl	3.0	3.0	3.0
Sugar beet pulp	10.0	10.0	10.0
Cholesterol	–	3.50	3.50
Coconut oil, hydrogenated	–	180	180
Soybean oil	21.0	2.0	5.0
Crude protein	168	168	167
Crude fat	42	200	200
Crude fiber	126	113	114
Crude ash	65	65	66
Starch	279	189	77
Sugar	38	32	176
Carbohydrates	471	363	379
Fatty acids			
C 6:0	–	0.6	0.6
C 8:0	–	9.9	9.9
C 10:0	–	9.3	9.3
C 12:0	–	81.9	81.9
C 14:0	0.2	35.4	35.4
C 16:0	6.3	22.9	22.7
C 18:0	1.6	21.9	22.0
C 20:0	0.2	0.3	0.3
C 16:1	0.3	0.1	0.1
C 18:1	8.7	5.3	5.6
C 18:2	21.9	10.0	9.8
C 18:3	3.7	2.3	2.2

[a] Vitamin & trace element content (addition per kg feed): 25.0 IU Vitamin A (E672), 1.50 IU, Vitamin D3 (E671), 0.125 g Vitamin E (all-rac-alpha-tocopherylacetate) (3a700), 0.08 g Vitamin K3 (MNB), 0.08 g Vitamin B1 (Thiamine mononitrate), 0.03 g Vitamin B2 (Riboflavin), 0.05 g Ca Pantothenate, 0.025 g Vitamin B6 (pyridoxol hydrochloride) (3a831), 0.00015 g Vitamin B12 (Cyanocobalamine), 0.09 g Niacin, 0.009 g Folic acid, 0.0005 g Biotin, 0.100 g Inositol, 0.100 g Iron (II)-sulfate monohydrate (E1), 0.005 Copper(II)-sulfate pentahydrate (E4), 0.03 g Manganese (II)-sulfate monohydrate (E5), 0.002 g Cobalt (II)-carbonate monohydrate (E3), 0.05 g Zinc sulfatemonohydrate (E6), 0.002 g Calcium iodate anhydrate (E2), 0.0001 g Sodium selenite (E8). CTRL: Control diet, HF: High fat diet, HFHS: High fat, high sucrose diet.

2.5. ELISA

BDNF analyses were carried out by a commercial ELISA kit (SEA011Gu, Cloud-Clone Corp, Houston, USA), as instructed by the manufacturer. All samples were diluted 1:20 in sterile PBS to obtain an

OD within the linear segment of the standard curve. All results were normalized to protein concentration.

Amyloid Precursor Protein (APP) in the hippocampus was analyzed by a commercial kit (EKA01603, Biomatik, Ontario, Canada), as instructed by the manufacturer. All samples were diluted 1:3 in sample diluent, as determined by the standard curve. All results were normalized to protein concentration. One animal was excluded for technical reasons.

2.6. Western blotting

Protein extracts from the frontal cortex and the hippocampus containing 10 µg protein were adjusted to a volume of 11.25 µl before adding 3.75 µl Laemmli Sample Buffer (Bio Rad, Hercules, CA, USA). Samples were denatured at 70 °C for 10 min and then transferred to a 7.5% Criterion™ TGX™ Precast Midi Protein Gel, (Bio Rad, Hercules, CA, USA). The electrophoresis was run for app. 40 min before protein transfer to a PVDF membrane (Bio Rad, Hercules, CA, USA) [47]. The following antibodies were applied: Synapsin I (ab8; Abcam, Cambridge, UK; 1:1000); CAMKII (Cba-2; Thermo Scientific, Waltham, MA, USA; 1:4000); phosphorylated CAMKII (p-CAMKII) (22B1; Thermo Scientific, Waltham, MA, USA; 1:1000); phosphorylated synapsin 1 (p-synapsin 1) (NB300-181; Novus Biologicals, Littleton, CO, USA; 1:1000); Anti-presenilin 1 (ab76083; Abcam, Cambridge, UK; 1:5000); anti-Neuronal nuclei protein (NeuN) (MAB377; Merck Millipore, Billerica, MA, USA; 1:2000) and anti-Glial Fibrillary Acidic Protein (GFAP) (ab7260; Abcam, Cambridge, UK; 1:20,000). All antibodies were incubated at 4 °C o/n. Fluorescent secondary antibodies were incubated for 1 h at RT: IRDye® 800CW Goat anti-Rabbit IgG (Li-Cor, Lincoln, NE, USA;1:15,000) and IRDye® 680RD Donkey anti-Mouse IgG (Li-Cor, Lincoln, NE, USA;1:15,000). All samples were run in duplicates. Calculations of fluorescence intensity were done by Image Studio 5.2 (Li-Cor, Lincoln, NE, USA) and samples normalized to total protein levels (REVERT™ Total Protein Stain, Li-Cor, Lincoln, NE, USA). One animal was excluded from hippocampal GFAP, NeuN and presenilin 1 analysis for technical reasons.

2.7. Statistics

The statistical analyses were performed in GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA) using one-way ANOVA. Bodyweight was analyzed using SAS enterprise Guide 7.1 (SAS Institute Inc, Cary, NC, USA) with a mixed linear model [19]. Spearman's correlation was computed to correlate the protein levels of total vitC, synaptic plasticity markers and levels of monoamines with the previously published plasma levels of triglycerides, cholesterol and low-density lipoprotein. In the event of inhomogeneous variances, the data was log-transformed and subsequently analyzed. Tukey's multiple comparisons test was used to correct for multiple comparisons. Results are presented as mean ± SD, median and ranges or geometric mean with 95% confidence intervals. A p-value < 0.05 was considered statistically significant.

3. Results

Bodyweight during the entire study period is shown in Fig. 1 [19]. CTRL animals displayed an increased weight gain compared to HF and HFHS animals and approached a plateau around week 16. HF and HFHS animals displayed a consistent increase, but remained below CTRL. At week 25 HF animals weighed less than CTRL (difference in mean bodyweight: 69.4 g equating to 7.8%, $p < 0.01$), whereas HFHS approached the weight of CTRL (difference in mean bodyweight: 38 g equating to 4.3%, $p = 0.068$). There was no difference in brain weight (results not shown) in HF and HFHS groups compared with CTRL. Plasma markers of dyslipidemia reflected dietary regimes (results shown as geometric mean with 95% confidence interval), as previously published [19]: Plasma total cholesterol (mM) CTRL: 1.15 [0.92–1.45], HF: 8.96 [7.17–11.30] and HFHS 9.38 [7.46–11.76]; plasma triglycerides (mM) CTRL 1.03 [0.80–1.31], HF: 0.46 [0.35–0.58] and HFHS: 0.46 [0.35–0.58]; and low-density lipoprotein (mM) CTRL: 1.22 [0.92–1.62], HF: 9.04 [6.81–12.0] and HFHS: 7.86 [5.90–10.4]. In all three markers of dyslipidemia, HF and HFHS differed significantly from CTRL ($p < 0.001$). A decrease in vitC levels (CTRL: 1132 ± 52.3 nmol/mg tissue, HF: 925 ± 142.2 nmol/mg tissue, HFHS: 969 ± 102.4 nmol/mg tissue) was evident in the frontal cortex (CTRL *vs.* HF, $p = 0.0094$; CTRL *vs.* HFHS $p = 0.0336$), but no differences between the experimental groups were detected in DHA and DHA%. Total vitC in the frontal cortex correlated significantly with plasma triglycerides ($p = 0.0039$, Spearman's $r = 0.6019$), low-density lipoprotein ($p = 0.0233$, Spearman's $r = -0.4925$) and cholesterol ($p = 0.0310$, Spearman's $r = -0.4714$).

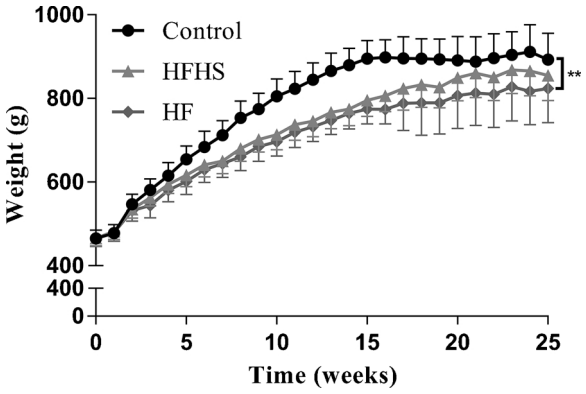

Fig. 1. Changes in bodyweight during the study period. After week 16, half of the animals in each group were euthanized and the remaining animals continued on their respective diets until week 25 (*i.e.* n = 14/group for week 0–15, and n = 7/group for week 16–25). There was a significant effect of time ($p < 0.001$), diet group ($p < 0.01$) and diet group*time interaction ($p < 0.001$). Data is depicted as mean ± SD, and was analyzed using a mixed linear model. The original data has previously been published [17].

In the hippocampus, no differences in either of the monoaminergic neurotransmitters, dopamine, norepinephrine and serotonin or the investigated metabolites were detected (Table 2). There was a significant increase in serotonin in frontal cortex in HF *vs.* CTRL ($p = 0.0023$) and HF *vs.* HFHS ($p = 0.0479$), while there was a tendency towards an increase in 5-hydroxyindoleacetic (5-HIAA) acid in HF animals, albeit not reaching significance (CTRL *vs.* HF $p = 0.0547$) (Table 2). BDNF was decreased in the hippocampus of HFHS (CTRL *vs.* HFHS $p = 0.0132$) (Fig. 2), while no changes were recorded in the frontal cortex. The ratio of p-CAMKII to CAMKII was significantly increased in the hippocampus of HF animals (CTRL *vs.* HF $p = 0.0415$) (Fig. 3), but no changes in any of the other plasticity markers were detected in the frontal cortex and the hippocampus.

No changes were observed in NeuN, GFAP, PSEN1 and APP (results not shown), but there was a tendency towards a decrease in PSEN1 in the HF hippocampus (CTRL *vs.* HF $p = 0.0763$).

In the frontal cortex, correlation analysis showed that the levels of triglycerides in plasma correlated significantly with 5-HIAA ($p = 0.0245$, Spearman's $r = -0.4888$), while low-density lipoprotein correlated with levels of 3,4-dihydroxyphenylacetic acid (DOPAC) ($p = 0.0422$, Spearman's $r = 0.447$), homovanillic acid (HVA) ($p = 0.0019$, Spearman's $r = 0.6381$) and 5-HIAA ($p = 0.0175$, Spearman's $r = 0.5127$), albeit did not reach significant correlation with serotonin ($p = 0.0536$, Spearman's $r = 0.4269$). Plasma cholesterol correlated significantly with DOPAC ($p = 0.0310$, Spearman's $r = 0.4714$), HVA ($p = 0.0017$, Spearman's $r = 0.6429$), 5-HIAA ($p = 0.0174$, Spearman's $r = 0.513$) and serotonin ($p = 0.0430$, Spearman's $r = 0.4455$) in the frontal cortex. No significant correlations were detected between dyslipidemia and the remaining neuronal

Table 2
The levels of monoaminergic neurotransmitters and selected metabolites in the frontal cortex and the hippocampus. Results are shown as mean ± SD or logarithmic mean (95% confidence interval). One-way ANOVA with Tukey's multiple comparison test, n = 7 for all samples.

		MHPG pmol/mg protein	NE pmol/mg protein	MHPG/NE	DOPAC pmol/mg protein	HVA pmol/mg protein	DA pmol/mg protein	HVA/DA	DOPAC/DA	5-HIAA pmol/mg protein	5-HT pmol/mg protein	5-HIAA/5-HT
Frontal Cortex	CTRL	5.48 ± 2.62	30.79 ± 5.62	0.08 (0.06–0.12)	10.47 (7.14–15.33)	7.39 (3.75–14.57)	10.81 (7.15–16.35)	0.68 (0.39–1.19)	0.97 (0.64–1.47)	15.46 (13.12–18.22)	46.79 ± 9.33	0.34 (0.29–0.39)
	HFHS	4.70 ± 0.54	35.08 ± 5.29	0.07 (0.05–0.10)	16.86 (11.51–24.70)	16.73 (8.49–32.99)	17.68 (11.69–26.74)	0.95 (0.54–1.65)	0.95 (0.63–1.44)	19.86 (16.85–23.40)	52.99 ± 6.24	0.38 (0.32–0.44)
	HF	5.39 ± 1.53	36.48 ± 7.74	0.09 (0.06–0.12)	16.79 (11.47–24.60)	15.23 (7.72–30.02)	13.15 (8.70–19.90)	1.16 (0.66–2.02)	1.28 (0.84–1.93)	20.39 (17.31–24.03)	64.14[a,b] ± 8.41	0.32 (0.27–0.38)
Hippocampus	CTRL	3.76 ± 1.16	42.61 ± 8.71	0.089 ± 0.026	8.56 (4.54–16.16)	7.25 (2.42–21.71)	0.92 (0.53–1.61)	1.38 ± 0.76	26.57 ± 6.54	6.70 (3.37–13.33)	64.49 ± 15.83	0.42 ± 0.09
	HFHS	2.90 ± 1.03	37.44 ± 12.37	0.083 ± 0.034	8.55 (4.53–16.13)	7.90 (2.64–23.63)	1.66 (0.95–2.90)	1.28 ± 0.52	27.60 ± 10.82	13.10 (6.58–26.05)	56.11 ± 11.04	0.49 ± 0.13
	HF	3.90 ± 0.86	43.28 ± 6.37	0.09 ± 0.009	7.11 (3.77–13.42)	6.18 (2.07–18.50)	1.79 (1.03–3.13)	1.24 ± 0.51	28.48 ± 10.80	11.08 (5.57–22.05)	58.44 ± 13.40	0.48 ± 0.12

MHPG: 3-Methoxy-4-hydroxyphenylglycol, NE: Norepinephrine, DOPAC: 3,4-dihydroxyphenylacetic acid, HVA: Homovanillic acid, DA: Dopamine, 5-HIAA: 5-hydroxyindoleacetic acid, 5-HT: Serotonin, CTRL: Control diet, HF: High fat diet, HFHS: High fat, high sucrose diet.

[a] CTRL *vs.* HF p = 0.0023.

[b] HFHS *vs.* HF p = 0.0478.

markers.

4. Discussion

This study reports, that high fat diet-induced chronic dyslipidemia decreases levels of vitC and increases serotonin in the frontal cortex, while increasing the p-CAMKII/CAMKII ratio in the hippocampus. The addition of sucrose to the HF diet abolishes the effect on serotonin in the frontal cortex and the p-CAMKII/CAMKII ratio in the hippocampus, while decreasing BDNF in the hippocampus. Furthermore, markers of dyslipidemia correlate with monoaminergic neurotransmitters and metabolites in the frontal cortex. Importantly, these changes occur in the absence of obesity, suggesting a direct effect of an adverse diet and the subsequent dyslipidemia on markers of brain function.

One of the mechanisms, through which Westernized diets have been shown to elicit their damaging effects, is by increasing oxidative stress [34,45]. Both HF and HFHS diets led to lower levels of vitC in the frontal cortex and markers of dyslipidemia directly correlated with total vitC in the frontal cortex, suggesting a direct effect of dyslipidemia on cerebral vitC levels. We have previously found a high fat diet to decrease both cerebral vitC and glutathione, another important antioxidant in the brain, in guinea pigs [15], while vitC deficiency itself causes redox imbalances and oxidative damage to lipids and DNA [29,36]. Thus, the decreases in cerebral vitC in the current study may indicate a disruption of cerebral redox balance. However, this could not be supported by a concurrent increase in DHA%. Low levels of vitC in the brain have been associated with memory dysfunction [50], dementia [11,54], and monoaminergic neurotransmitter changes [32,51] in addition to increased oxidative stress [16,36], supporting vitC as a crucial component in maintaining normal brain function. However, vitC serves both as an unspecific antioxidant and a co-factor in specific reactions such as the conversion of dopamine to norepinephrine [27] and has been proposed to take an active part in preventing glutamate-induced excitotoxicity [39]. It is possible, that an effect of low brain vitC levels in the current study would affect these more specific pathways as opposed to overall redox imbalance. In the hippocampus, BDNF levels appeared to be decreasing with dyslipidemia, albeit only reaching significance in the HFHS group. Other studies have shown a negative effect on BDNF expression following diets high in sugar and/or fat, but most commonly in association with increased bodyweight [33,46], which is not a feature of our model. The absence of obesity is characteristic of the diet-induced dyslipidemic guinea pig model and the recorded differences are likely to be reflecting a slight delay in weight-gain in high fat fed animals [49]. We don't know the reason for this, but speculate that there may be a difference in preference between the diets, inducing a higher intake of hay in the high fat fed animals. At termination, groups are approaching similar levels in body weight in coherence with the expected plateau of mature adult animals at this age and supporting that the welfare of animals in HF and HFHS groups is unaffected.

Despite a lower level of BDNF in the hippocampus of the HFHS group, no additional differences in the selected plasticity markers were detected. Whether this is due to BDNF levels still being sufficiently high to maintain downstream targets or that other BDNF regulated pathways are affected remains to be determined.

However, in the HF group, an increase in the p-CAMKII/CAMKII ratio was detected in the hippocampus. Autophosphorylation of threonine-286 gives the kinase an autonomous activity, independent of Ca^{2+} levels, and is a key component of long-term potentiation and memory formation [17]. The HF diet-imposed increase in the ratio of autophosphorylated CAMKII could be interpreted as a beneficial effect of HF on this plasticity marker. However, as a part of the p-CAMKII/CAMKII increase is due to an overall decrease in total CAMKII, it may be that the increased ratio is the result of combined effects. Taken together, our findings indicate that dyslipidemia altered total CAMKII, potentially imposing a negative effect on long term potentiation and

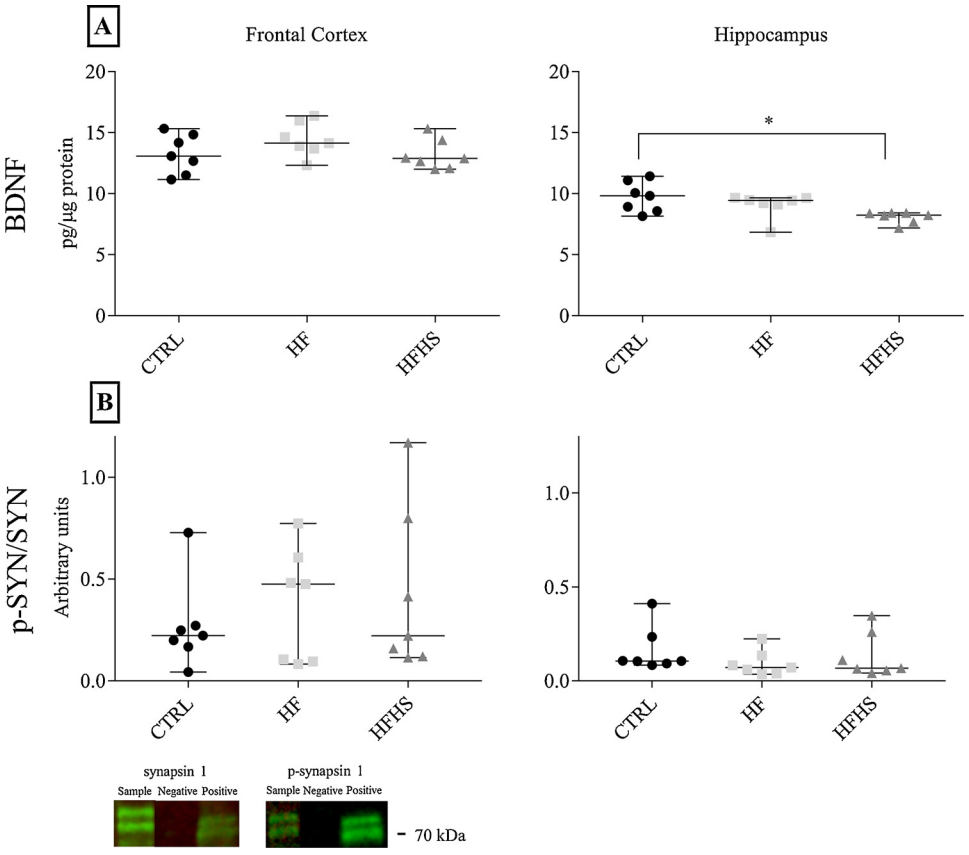

Fig. 2. Levels of selected molecular markers in the frontal cortex and the hippocampus. (A) BDNF in the frontal cortex and the hippocampus. Hippocampus: CTRL *vs.* HFHS $p = 0.0132$. (B) p-synapsin 1 to synapsin 1 ratio in the frontal cortex and the hippocampus. All values are presented as median and range and were analyzed by one-way ANOVA with Tukey's multiple comparison test, $n = 7$.*: $p < 0.05$. BDNF: Brain-derived neurotrophic factor; SYN: synapsin 1; p-SYN: phosphorylated synapsin 1; CTRL: Control diet, HF: High fat diet, HFHS: High fat, high sucrose diet. Pictures of representative Western blot bands are included to confirm band specificity. The two bands seen at 70 and 74 kDa on the synapsin and p-synapsin blots are consistent with splice variants.

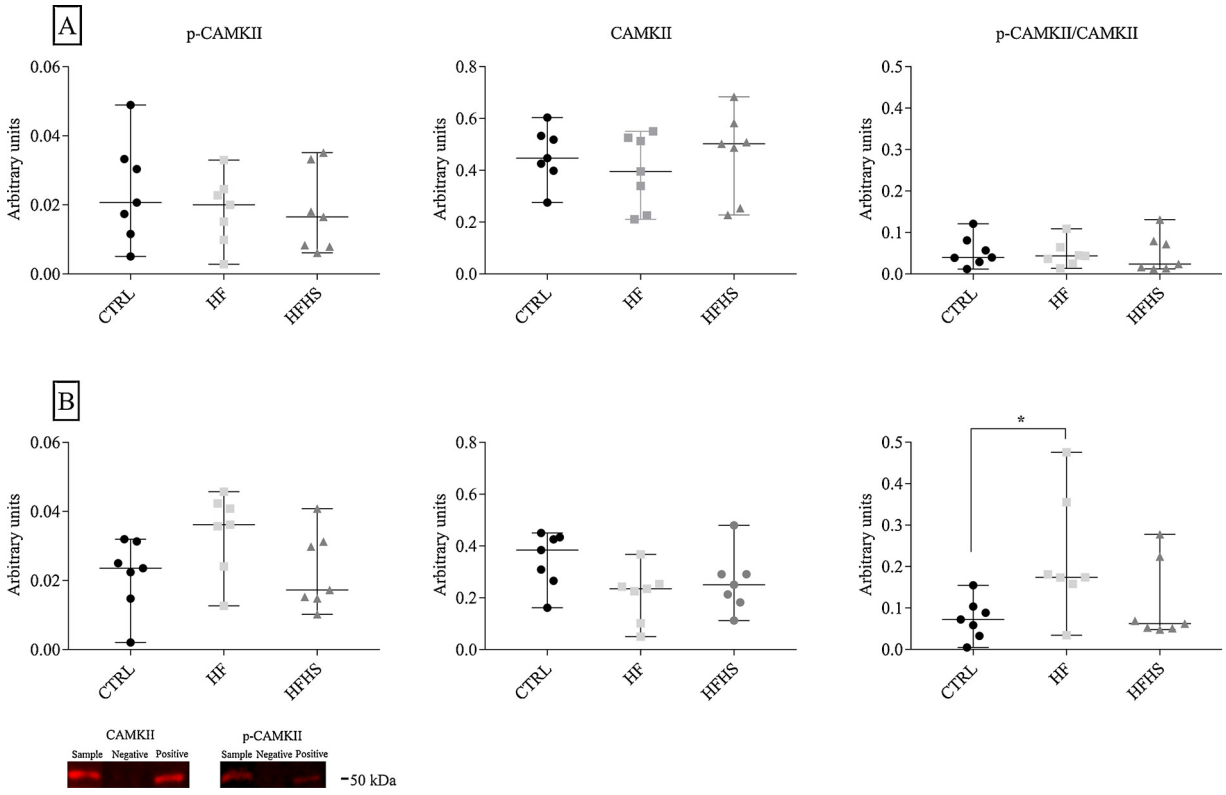

Fig. 3. Levels of p-CAMKII, CAMKII and the ratio in the frontal cortex and the hippocampus. p-CAMKII to CAMKII ratio in the hippocampus; CTRL *vs.* HF, $p = 0.0415$. All values are presented as median and range and were analyzed by one-way ANOVA with Tukey's multiple comparison test, $n = 7$.*: $p < 0.05$. CTRL: Control diet, HF: High fat diet, HFHS: High fat, high sucrose diet; CAMKII: Ca^{2+}/calmodulin-dependent protein kinase II; p-CAMKII: phosphorylated Ca^{2+}/calmodulin-dependent protein kinase II. Pictures of representative Western blot bands are included to confirm band specificity.

synaptic plasticity that subsequently induced a compensatory response by increasing CAMKII-activation through phosphorylation. HF also increased the levels of serotonin in the frontal cortex. Treatment with selective serotonin reuptake inhibitors has previously been shown to increase BDNF levels [1,14] and it may be that the increased serotonin levels of the frontal cortex in the HF is a compensatory response to maintain BDNF levels at normal. This, however, remains to be confirmed.

Correlation analysis showed that dyslipidemia influenced dopaminergic and serotonergic neurotransmitters and their metabolism (correlation with 5-HIAA, serotonin, DOPAC and HVA) in the frontal cortex. Dyslipidemia was also correlated with levels of total vitC in the frontal cortex. VitC is involved in the synthesis of the monoaminergic neurotransmitters [13,30] and deficiency has been found to affect their synthesis and metabolism [32,51]. This supports, that dyslipidemia may affect levels of monoaminergic neurotransmitters by reducing VitC levels. The effect of dyslipidemia on the monoaminergic neurotransmitters was confined to the frontal cortex, a brain area critical in working memory, higher executive functions and mood [44], rendering imbalances in neurotransmitter function to possibly impact these functions. Working memory functions have been found to be impacted directly by Westernized diets [22], primarily through effects on hippocampus-dependent cognitive functions [33,46,52].

Western diets have been shown to exacerbate the development of dementia and Alzheimer's disease in animal models and humans [18,40,48]. Guinea pigs have similarities with human APP sequence and processing and genes associated with Alzheimer's disease have been shown to increase during a high cholesterol dietary regime [2,43]. However, despite a tendency towards decreased presenilin 1 in the HF group, we did not find significant changes in the investigated markers. This was also the case in the current correlation analysis, where hippocampal PSEN1 and APP approached, but did not reach significant correlation, with markers of dyslipidemia. This could be due to the relatively young age of our animals, 35 weeks old at study termination. Considering a lifespan of 5–6 years, these animals were still young adults, well away from old age and senescence most commonly associated with this type of neurodegenerative disorder.

In conclusion, this study reports that diet-induced dyslipidemia affects antioxidant levels, neurochemistry and molecular markers in a region-specific manner in the brain. Interestingly, these changes occurred in the absence of obesity, suggesting that these effects are a direct, rather than indirect, consequence of a high fat diet and the associated dyslipidemia.

Conflicts of interest

The authors declare no conflicts of interest.

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