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ORIGINAL ARTICLE

Exercise training and high-fat diet elicit endocannabinoid system modifications in the rat hypothalamus and hippocampus

François-Xavier Gamelin[1,2] · **Julien Aucouturier**[1,2] · **Fabio Arturo Iannotti**[3] · **Fabiana Piscitelli**[3] · **Enrico Mazzarella**[3] · **Teresa Aveta**[3] · **Melissa Leriche**[1,2] · **Erwan Dupont**[1,2] · **Caroline Cieniewski-Bernard**[1,2] · **Erwan Leclair**[1,2] · **Bruno Bastide**[1,2] · **Vincenzo Di Marzo**[3] · **Elsa Heyman**[1,2]

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Abstract The purpose of the present study was to examine the effect of chronic exercise on the hypothalamus and hippocampus levels of the endocannabinoids (eCBs) anandamide (AEA) and 2-arachidonoylglycerol (2-AG) and of two AEA congeners and on the expression of genes coding for CB1, CB2 receptors (*Cnr1* and *Cnr2*, respectively), and the enzymes responsible for eCB biosynthesis and degradation, in rats fed with a standard or high-fat diet. Male Wistar rats (n = 28) were placed on a 12-week high-fat (HFD) or standard diet period, followed by 12 weeks of exercise training for half of each group. Tissue levels of eCBs and related lipids were measured by liquid chromatography mass spectrometry, and expression of genes coding for CB1 and CB2 receptors and eCB metabolic enzymes was measured by quantitative real-time polymerase chain reaction (qPCR). HFD induced a significant increase in 2-AG ($p < 0.01$) in hypothalamus. High-fat diet paired with exercise training had no effect on AEA, 2-AG, and AEA congener levels in the hypothalamus and hippocampus. *Cnr1* expression levels were significantly increased in the hippocampus in response to HFD, exercise, and the combination of both ($p < 0.05$). Our results indicate that eCB signaling in the CNS is sensitive to diet and/or exercise.

Keywords 2-arachidonoylglycerol · Anandamide · CB1 receptor · CB2 receptor · Hippocampus · Hypothalamus

Introduction

Strong evidence supports a role of the endocannabinoid system (ECS) in the impaired regulation of food intake and energy metabolism that contribute to the development of obesity [15, 43]. The ECS is a complex endogenous signaling system comprising 7-transmembrane domain receptors (cannabinoid type 1 (CB1) and type 2 (CB2) receptors), their endogenous lipid-derived ligands (the endocannabinoids, eCBs), and enzymes for eCB biosynthesis and degradation. The two most studied eCBs are *N*-arachidonoylethanolamine (AEA), also known as anandamide, and 2-arachidonoylglycerol (2-AG). eCBs are not stored in cells but are synthesized on demand from arachidonic acid containing phospholipid precursors through enzyme activation by multiple pathways in the cell membrane of most mammalian cells such as neurons, adipocytes, and skeletal muscle cells, possibly in response to elevated levels of intracellular calcium [6], membrane depolarization, and/or receptor stimulation [36].

In the central nervous system (CNS), the ECS interacts with the different systems involved in control of food intake and energy expenditure [15]. In rodents, AEA injection in hypothalamus elicits increased feeding via CB1 activation [27] by modulating probably the expression and the action of orexigenic and anorectic mediators, such as the neuropeptide melanin-concentrating hormone or the corticotropin-releasing hormone [9, 28]. In addition to the regulation of food

Vincenzo Di Marzo, and Elsa Heyman share the senior authorship.

✉ François-Xavier Gamelin
francois-xavier.gamelin-2@univ-lille2.fr

[1] Univ Lille Nord de France, F-59000, Lille, France

[2] Equipe d Accueil 7369, URePSSS - Unité de Recherche Pluridisciplinaire Sport Santé Société - Equipe Activité Physique, Muscle, Santé, Eurasport, 413 rue Eugène Avinée, 59120 Loos, France

[3] CNR, Endocannabinoid Research Group, Institute of Biomolecular Chemistry, I-80078 Pozzuoli, Italy

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intake, the hypothalamic ECS is also involved in energy expenditure regulation by acting on thermogenesis [29].

The ECS also plays a role in hedonic aspect of food intake by modulating brain area activities of the reward system such as the nucleus accumbens, ventral tegmental area, or hippocampus [31] [34]. Kirkham et al. [30] observed that the injection of 2-AG into the nucleus accumbens shell produced a short-term stimulatory action on feeding behavior in free-feeding rats. The ECS seems to facilitate the mesolimbic dopamine signaling that stimulates appetite, as Verty et al. [49] observed that a dopamine D1 receptor antagonist attenuates feeding induced by a CB1 agonist.

Previous studies in obese rodents suggest the presence of ECS dysregulation in the hypothalamus and hippocampus [14, 34]. Di Marzo et al. [14] found that genetically obese rats and mice with disrupted leptin signaling (Zucker *fa/fa* rats and *db/db* mice), as well as mice lacking leptin (*ob/ob* mice), have higher hypothalamic endocannabinoid levels compared with wild-type animals. Similar findings were also reported in the hippocampus of mice with diet-induced obesity [34]. These central ECS alterations, together with peripheral eCB disturbances in the adipose tissue and skeletal muscle, may participate in excessive and/or ectopic fat accumulation and related metabolic disorders [12, 35].

In human obesity, ECS dysregulation is supported by the observation of changes in AEA and/or 2-AG levels in the plasma and adipose tissue [3, 18]. The ECS represents a primary target for the treatment of abdominal obesity and associated metabolic changes, whether its dysregulation is a consequence or a cause of obesity. It is noteworthy that before being withdrawn from the market due to psychiatric side effects such as anxiety and depression [8], CB1 antagonists were clearly shown to be effective at reducing body weight and waist circumference in obese subjects [38].

Exercise is a recognized treatment of obesity [37]. One bout of exercise triggers eCB signaling by elevating AEA plasma levels [45] [23, 40]. However, there is some evidence suggesting that a more long-lasting healthy lifestyle approach may be effective to reverse ECS dysregulation [3, 17] and may represent a safe alternative to the pharmacological approach. A 1-year lifestyle modification program including physical activity induced a significant decrease in fasting plasma AEA (−7.1%) and, most importantly, 2-AG (−62.3%) levels in viscerally obese men [17]. In subcutaneous and visceral adipose tissues, chronic exercise limits CB1 gene expression increase induced by high-fat diet in rodents [51]. Thus, chronic exercise may counteract ECS dysregulation in these tissues.

In the CNS, the ECS mediates exercise-induced hippocampus plasticity [25] and reward [20]. However, it remains unknown whether exercise has beneficial effects on the ECS in the CNS of obese animals, and more specifically the hypothalamus and hippocampus, involved in the control of energy balance [32, 48].

This study aimed therefore at identifying potential changes elicited by exercise training in the brain ECS of rats on a high-fat diet, compared to rats on a standard diet. With this purpose, we determined the hypothalamic and hippocampal levels of AEA, 2-AG, and two AEA congeners *N*-oleylethanolamine (OEA) and *N*-palmitoyl-ethanolamine (PEA); the expression of genes encoding for eCB receptors (CB1, CB2) and enzymes involved in eCB anabolic (α/β-hydrolase 4, glycerophosphodiesterase 1, N-acylphosphatidylethanolamine-phospholipase D, protein tyrosine phosphatase N22 (respectively ABHD4, GDE-1, NAPE-PLD, and PTNP-22) for AEA, OEA, and PEA; diacylglycerol lipase α (DAGL-α) and diacylglycerol lipase β (DAGL-β), for 2-AG) and catabolic (fatty acid amide hydrolase (FAAH), for AEA, OEA, and PEA; α/β-hydrolase 6 (ABHD6), α/β-hydrolase 12 (ABHD12), and monoacylglycerol lipase (MAGL), for 2-AG) pathways [16]. We also determined the expression of the transient receptor potential vanilloid type-1 (TRPV1) channel, which is activated by eCBs and AEA congeners and is an ionotropic receptor for eCBs [52].

Materials and methods

Animals and general procedure

General procedures were already described previously [21]. Briefly, 28 male Wistar rats (3 weeks old) were housed in groups of two or three per cage. After 1 week of acclimatization, rats were randomly divided into two groups and fed ad libitum either with a standard diet (energy equivalent: 2.90 kcal g^{-1}) or a high-fat diet (energy equivalent 5.05 kcal g^{-1}) during 24 weeks. After 12 weeks, half of the rats were submitted to 12 weeks of exercise training (Ctl + training and HFD + training). The second half of the rats remained untrained for 12 weeks (Ctl and HFD groups for rats on standard and high-fat diets, respectively). Before and after the training period, Ctl + training and HFD + training performed a maximal aerobic velocity (MAV) test on the treadmill. Five days before sacrifice, all rats were subjected to an oral glucose tolerance test (OGTT). At the end of the exercise training period, blood samples were collected by cardiac puncture, after which all rats were euthanized and the hypothalamus and hippocampus removed.

All procedures described were approved by the Agricultural and Forest Ministry and the National Education Ministry (Veterinary service of health and animal protection) and were in accordance with the European Union Directive of 22 September 2010 (2010/63/UE).

High-fat diet

Rats were fed with two different types of diet during the 24 weeks of the experimentation:

- A high-fat diet (Purified Diet 231 HF, Safe, Augy, France) with an energy equivalent of 5.05 kcal g^{-1} and containing 26.9% of proteins, 39.7% of lipids, and 10.1% of carbohydrates
- A standard diet with an energy equivalent of 2.90 kcal g^{-1}. It contained 16% of proteins, 3% of lipids, 60% of carbohydrate, and 21% of other components (fiber, mineral, humidity).

Fatty acid compositions of the two diets are described in Table 1. Food and caloric intake by each rat and their weight gain were estimated two times per week during the experimentation.

Table 1 Fatty acid compositions of the standard (Ctl) and the high fat diet (HFD)

	Ctl	HFD
Total fat (g/kg)	27.50	395.47
Total saturated fat (g/kg)	6.34	140.36
C10:0	–	0.32
C12:0	0.03	0.32
C14:0	0.17	4.19
C15:0	0.03	0.32
C16:0	5.30	86.93
C17:0	0.03	1.29
C18:0	0.58	45.97
C20:0	0.10	0.94
C22:0	0.06	0.07
C24:0	0.06	–
Total monounsaturated fat (g/kg)	5.61	171.18
C16:1	0.19	8.14
C17:1	0.03	0.65
C18:1	5.09	158.96
C19:1	–	0.00
C20:1	0.30	2.80
C22:1	–	0.65
Total polyunsaturated fat (g/kg)	15.57	86.54
C18:2	13.70	78.14
C18:3	1.13	3.24
C18:4	–	2.58
C20:2	0.03	1.29
C20:3	–	0.65
C20:4	0.06	–
C20:5	0.14	–
C22:1	0.19	–
C22:4	–	0.32
C22:5	0.06	0.32
C22:6	0.22	–
C24:1	0.06	–
Total ω3 fatty acids (g/kg)	1.57	3.91
Total ω6 fatty acids (g/kg)	13.70	75.35

Maximal aerobic velocity test

Animals of in the HFD + training and Ctl + training were familiarized with treadmill running (L810, Bioseb. France) for 10 min for 5 days at a velocity of 20 cm s^{-1} and a 0° slope. Electric shocks (intensity <1.2 mA) were used to motivate the rat to run. After the familiarization period, Ctl + training and HFD + training groups performed a graded exercise test to voluntary exhaustion. The test started at 20 cm s^{-1} for 5 min, followed by speed increment of 3 cm s^{-1} every 3 min until the animal could no longer keep up with the treadmill speed. Exhaustion was reached when animal sat longer than 10 s on electric shock grid. MAV was defined as the velocity of the last 3-min stage completed. The same protocol was repeated 1 week before rats sacrifice to determine the change in MAV with exercise training (2 days before the OGTT).

Exercise training

The day after the baseline MAV test, Ctl + training and HFD + training groups started the 12-week exercise training period that consisted of treadmill running for 1 h/day, 5 days/week at an intensity set between 70 and 80% of the MAV. The intensity was increased by 1 cm s^{-1} every week to take into the adaptations to exercise training. Animal exercised at the same hour of the day at the end of the room dark cycle (7:30 a.m.). Control groups were in the same room during the training session and handled in the same way to induce a similar stress level. Three days before the sacrifice, exercise training was stopped to avoid the confounding fatigue or stress effect of acute exercise.

Oral glucose tolerance test

Five days before sacrifice, the animals were fasted overnight. Basal blood glucose level, defined as T0, was determined using an automatic glucometer (Accu-Chek Performa; Roche Diagnostics) before oral administration (4 ml kg^{-1} of body weight) of a D-glucose solution (50%). Tail vein blood glucose was then measured at 30, 60, 90, and 120 min after the administration. Total area under the curve (AUC) was calculated using the trapezoidal method [39].

Sample collection

The day before the end of the experiment, rats were fasted in order to obtain the same nutritional state for each. Animals were anesthetized with pentobarbital sodium (60 mg kg^{-1} of body weight, i.p.), and blood samples were collected by cardiac puncture. Samples were drawn directly into pre-cooled 5-mL EDTA tubes. EDTA blood was immediately centrifuged (less than 5 min after sampling), and plasma was removed and frozen (−80 °C) until analysis. Then, the rats were sacrificed by decapitation. The head was immediately surrounded with ice, and the hypothalamus and hippocampus were quickly removed, weighed, and immediately frozen in liquid nitrogen. Then, they were stored at −80 °C until analyses.

Plasma analyses

Fasting levels of glucose and insulin were determined in plasma. The content of glucose in plasma was measured using a commercially available colorimetric assay kit (Cayman Chemical Company, USA). Plasma insulin was determined using a commercially available rat insulin enzyme immunoassay kit (SPI-BIO, France).

Measurements of endocannabinoids

The extraction, purification, and quantification of EC from tissues have been performed as previously described [26]. Briefly, the tissues were dounce-homogenized and extracted with chloroform/methanol/Tris–HCl 50 mmol l^{-1} pH 7.5 (2:1:1, vol/vol) containing internal standards ($[^2H]_8$ AEA; $[^2H]_5$ 2-AG, $[^2H]_5$ PEA, and $[^2H]_4$ OEA, 5 pmol each). The lipid-containing organic phase was dried down, weighed, and pre-purified by open-bed chromatography on silica gel. Fractions were obtained by eluting the column with 99:1, 90:10, and 50:50 (*v*/*v*) chloroform/methanol. The 90:10 fraction was used for AEA, 2-AG, PEA, and OEA quantification by liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry by using a Shimadzu high-performance liquid chromatography apparatus (LC-10ADVP) coupled to a Shimadzu (LCMS-2020) quadruple mass spectrometry via a Shimadzu atmospheric pressure chemical ionization interface as previously described [26]. The amounts of analytes in tissues, quantified by isotope dilution with the above-mentioned deuterated standards, were expressed as picomole per gram or milligram of wet tissue weight.

RNA purification and qPCR

Total RNA was isolated from native tissues by use of the TRI-Reagent (Sigma-Aldrich, Milan, Italy), reacted with DNase-I (1 U/ml; Sigma-Aldrich) for 15 min at room temperature, and followed by spectrophotometric quantification. Final preparation of RNA was considered DNA- and protein-free if the ratio between readings at 260/280 nm was ≥1.7. Isolated mRNA was reverse transcribed by use of SuperScript III Reverse Transcriptase (Life Technologies, Monza (MI), Italy). The quantitative real-time PCR was carried out in CFX384 real-time PCR detection system (Bio-Rad, Segrate (MI), Italy), with specific primers [26], by the use of SYBR Green master mix kit (Bio-Rad, Segrate (MI)) (see Table 2 for primer sequence). Samples were amplified simultaneously in quadruplicate in one-assay run with a non-template control blank for each primer pair to control for contamination or primer-dimer formation, and the cycle threshold (ct) value for each experimental group was determined. The housekeeping gene (the hypoxanthine-guanine phosphoribosyltransferase, hprt) was used as an internal control to normalize the ct values, using the $2^{-\Delta Ct}$ formula; differences in mRNA content between groups were as expressed as $2^{-\Delta\Delta Ct}$.

Statistical analyses

Data are shown as means ± SE. Normal Gaussian distribution of the data was verified by the Shapiro-Wilk test. Repeated measure analysis of variance (ANOVA) was used to evaluate the evolution of weight and accumulated caloric intake during the first 12 weeks of the experiment, the evolutions of weight and MAV during the exercise-training period and mean caloric intake of the next to last week of each period, and the glycemia during the OGTT. Multiple comparisons were made with the Newman-Keul post hoc test. A two-way ANOVA was used to evaluate the effects of diet, exercise training, and the diet–exercise interaction on metabolic parameters (fasting glucose, insulin, AUC during OGTT) and on eCBs and congeners tissue levels. Multiple comparisons were made with the Bonferroni post hoc test if significant main effects or interaction were observed with ANOVA. Concerning gene expression data, the Ctl group was compared with other groups by using Student's *t* test. Statistical significance was set at $p = 0.05$ level for all analysis. All calculations were made with Statistica 6.0 (Statsoft, Tulsa, USA).

Results

Effect of diet and/or exercise on body mass, caloric intake, maximal aerobic velocity, basal glucose and insulin levels, and glucose tolerance

Figure 1 shows body weight gain over the diet and exercise training period. Over the first 12 weeks, body mass increased with time and this is all the more in the case of the high-fat diet (Table 3). From the 10th week, rats fed with the high-fat diet were significantly heavier than were the rats fed with the

Table 2 Primers sequence used in qPCR analysis

Gene	Forward sequence (5′-3′)	Reverse sequence (5′-3′)	Enter accession number	Product length (bp)
abdh$_{12}$	CAGGCGTGCGGTCGAAACCA	TCAAGCTGCAGTCGGCGTCC	NM_001024314.1	189
abdh$_4$	TCTGGCGTCAAGCGGAGGGA	ACGCCACCCCCAAAGCCATG	NM_001108866.1	299
abdh$_6$	AGCGTCTGCTCCCATCCCCA	TGGCTTGCCAGTGGCGTGAA	NM_001007680.1	255
cnr-1	CTGAGGGTTCCCTCCCGGCA	TGCTGGGACCAACGGGGAGT	NM_012784.4	285
cnr-2	GCGGCTAGACGTGAGGTTGGC	TCCTTCAGGACCAAGAGTCTCAGCCT	NM_020543.4	335
daglα	GGCCGCACCTTCGTCAAGCT	ATCCAGCACCGCATTGCGCT	NM_001005886.1	380
daglβ	AGACCCGGGTGCAATGCTGC	GCCCTGGTGTGTGGGTCACG	NM_001107120.1	212
faah	GGCAGAGCCACAGGGGCTATCA	TGGGGCTACAGTGCACAGCG	NM_024132.3	349
gde-1	GCAGCCCCTTCAACGCCTGT	GATGGCCGCCAGCGTGTTCT	NM_019580.4	172
magl	CGGAACAAGTCGGAGGTTGA	TGTCCTGACTCGGGGATGAT	NM_138502.2	220
nape-pld	AGGCTGGCCTACGAATCACGT	ATGGTACACGGGGGACGGCG	NM_199381.1	150
ptpn-22	TGGTCGTGGGAGAGCCGCTT	GGGCCACTTTTTGCGCCTGC	NM_001106460.1	263
trpv1	AGACATCAGCGCCCGGGACT	CCAGCTTCAGCGTGGGGTGG	NM_031982.1	151

control diet ($p < 0.01$; Fig. 1). During the exercise period, rats fed with the high-fat diet continued to gain more weight with time while exercise training slowed down the time-induced body gain, especially for the HFD + training group (Table 3).

Accumulated caloric intake is described in Fig. 2. Over the first and second periods of the protocol, we observed that rats fed on the high-fat diet accumulated more and more rapidly calorie than did rats fed on the standard diet (Table 3). From the 8th week, rats fed with the high-fat diet accumulated significantly more calorie than did rats fed with the control diet ($p < 0.01$; Fig. 2). However, over the second period, the accumulated caloric intake was slowed down by exercise training (Table 3).

MAV was measured only in the exercise-trained groups to avoid familiarization in the Ctl and HFD groups that could affect the results. Two-way ANOVA for MAV revealed significant effects of diet and time but no significant interaction between both factors (Table 4). There were no significant differences for fasting plasma insulin levels between groups. Fasting plasma glucose concentration was increased by the high-fat diet but this increase was strongly reduced by exercise training (Table 4). Between-group comparison indicated that in the HFD group, glycemia was significantly higher than in the other groups (Table 4). Glucose AUC during the OGTT performed 1 week before the animal sacrifice was also significantly increased by the high-fat diet (Table 4) but without significant protecting

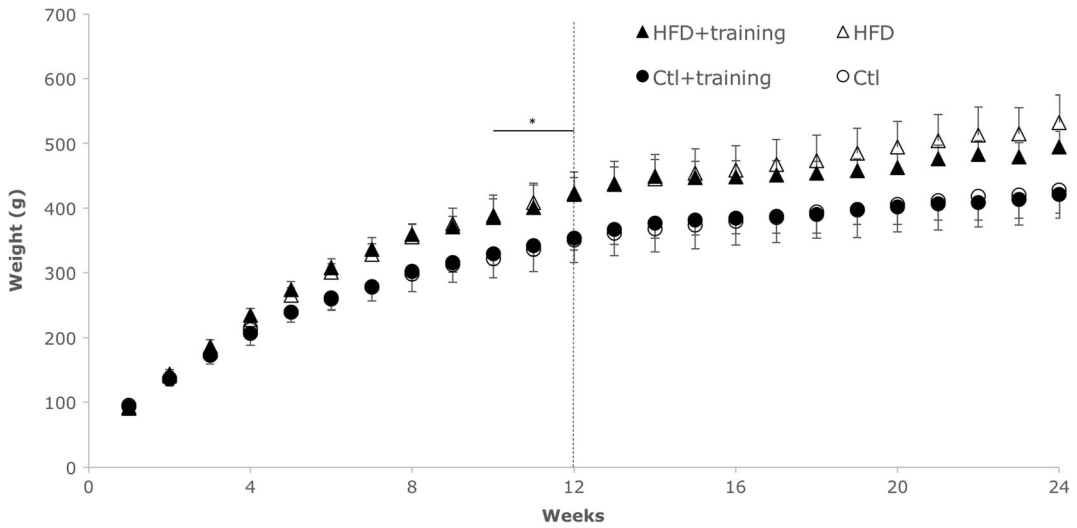

Fig. 1 Evolution of body weight during the 24 weeks of the protocol in control (ctl), high-fat diet (HFD), control with chronic exercise (ctl + training), and high-fat diet with chronic exercise (HFD + training) groups. The *dashed line* corresponds to the introduction of exercise in ctl + training and HFD + training groups. *Significant difference between rats fed with high-fat and standard diet during the first period of the protocol: $p < 0.01$

Table 3 F-statistic and its associated degrees of freedom and *p* value of analyses of variance used to compare the effects of diet and/or exercise on body weight; food intake; maximal aerobic velocity (MAV); basal glucose and insulin levels; and oral glucose tolerance test in control, high-fat diet, control with chronic exercise, and high-fat diet with chronic exercise groups

Weight (g)	Main effects by ANOVA	*F* values	*p*
During diet period	Diet	$F_{(1, 26)} = 53.80$	0.00001
	Time	$F_{(11, 297)} = 1714.96$	0.00001
	Time × Diet	$F_{(11, 286)} = 30.56$	0.00001
During exercise period	Diet	$F_{(1, 26)} = 38.91$	0.00001
	Ex	$F_{(1, 26)} = 0.60$	NS
	Diet × Ex	$F_{(1, 24)} = 0.59$	NS
	Time	$F_{(12, 288)} = 392.85$	0.00001
	Time × Diet	$F_{(12, 312)} = 5.34$	0.00001
	Time × Ex	$F_{(12, 312)} = 16.72$	0.00001
	Time × Diet × Ex	$F_{(12, 288)} = 3.48$	0.0001
Accumulated caloric intake (kcal)			
During diet period	Diet	$F_{(1, 26)} = 63.94$	0.00001
	Time	$F_{(11, 297)} = 9136.67$	0.00001
	Time × Diet	$F_{(11, 286)} = 71.46$	0.00001
During exercise period	Diet	$F_{(1, 26)} = 61.06$	0.00001
	Ex	$F_{(1, 26)} = 0.42$	NS
	Diet × Ex	$F_{(1, 24)} = 1.28$	NS
	Time	$F_{(10, 270)} = 12{,}597.78$	0.00001
	Time × Diet	$F_{(10, 260)} = 28.08$	0.00001
	Time × Ex	$F_{(10, 260)} = 2.99$	0.01
	Time × Diet × Ex	$F_{(10, 260)} = 0.28$	NS

The main effects from ANOVA are as follows: *Time* time effect, *Diet* diet effect, *Ex* exercise training effect, × interaction between variables, *NS* not significant

effect from exercise training. Regarding glucose kinetics during the OGTT (Fig. 3), the ANOVA revealed a significant effect of diet ($F_{(1, 26)} = 85.23$, $p < 0.006$) and interactions between time and diet ($F_{(4, 104)} = 3.97$, $p < 0.008$) as well time, training, and diet ($F_{(4, 96)} = 6.59$, $p < 0.0001$). The HFD group showed higher level of blood glucose than did other groups at 30 min ($p < 0.02$). At 90 and 120 min, blood glucose levels for the HFD groups were higher than for the Ctl + training group ($p < 0.05$ for all).

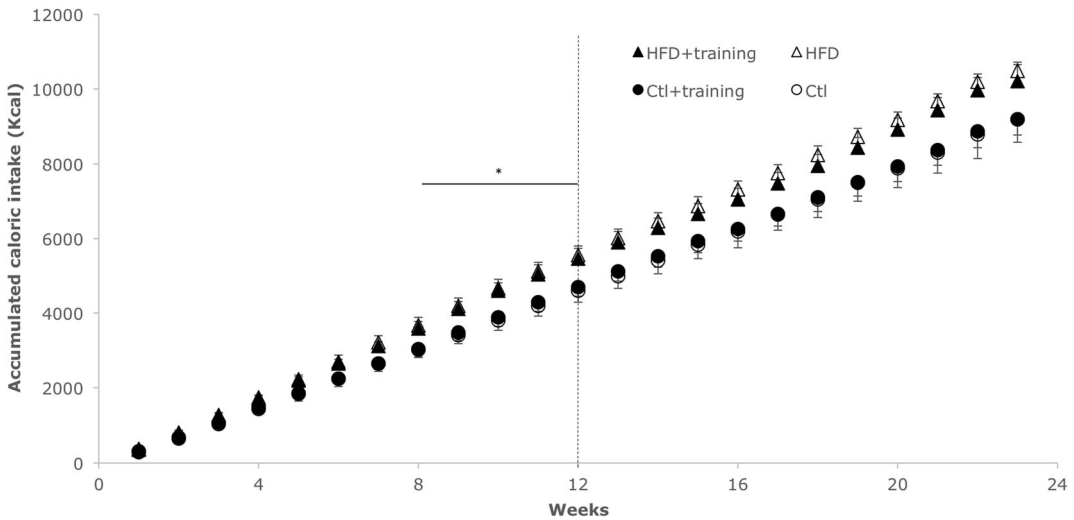

Fig. 2 Accumulated caloric intake during the 24 weeks of the protocol in control (ctl), high-fat diet (HFD), control with chronic exercise (ctl + training), and high-fat diet with chronic exercise (HFD + training) groups. The *dashed line* corresponds to the introduction of exercise in ctl + training and HFD + training groups. *Significant difference between rats fed with high-fat and standard diet during the first period of the protocol: $p < 0.01$

Table 4 Effect of diet and/or exercise on body weight; food intake; maximal aerobic velocity (MAV); basal glucose and insulin levels; and oral glucose tolerance test (OGTT) in control (Ctl), high-fat diet (HFD), control with chronic exercise (Ctl + training), and high-fat diet with chronic exercise (HFD + training) groups

	Ctl	HFD	Ctl + training	HFD + training	Main effects by ANOVA	*F* values	*p*
MAV (cm s^{-1})			45.9 ± 5.2	41.6 ± 3.9	Time	$F_{(1, 13)} = 95.80$	0.0001
During diet period			61.9 ± 6.0	55.8 ± 5.0	Diet	$F_{(1, 12)} = 5.41$	0.05
During exercise period					Time × Diet	$F_{(1,12)} = 0.36$	NS
Plasma insulin (ng ml^{-1})	2.8 ± 0.9	2.9 ± 0.5	2.2 ± 0.8	2.5 ± 0.3	Diet	$F_{(1, 26)} = 0.46$	NS
					Ex	$F_{(1, 26)} = 2.19$	NS
					Diet × Ex	$F_{(1, 24)} = 0.08$	NS
Plasma glucose (mg dL^{-1})	83 ± 3	103 ± 12*	86 ± 5	92 ± 5	Diet	$F_{(1, 26)} = 21.09$	0.0001
					Ex	$F_{(1, 26)} = 2.91$	NS
					Diet × Ex	$F_{(1, 24)} = 5.70$	0.05
OGTT (AUC)	247.3 ± 12.4	306.7 ± 20.4	247.7 ± 16.5	286.1 ± 9.8	Diet	$F_{(1, 26)} = 71.07$	0.0001
					Ex	$F_{(1, 26)} = 3.02$	NS
					Diet × Ex	$F_{(1, 24)} = 3.26$	NS

Data are means ± SD

The main effects from ANOVA are as follows: *Time* time effect, *Diet* diet effect, *Ex* exercise training effect, × interaction between variables

*Significantly different from all groups, $p < 0.05$

Effect of diet and/or exercise on AEA, 2-AG, PEA, and OEA levels and the expression of genes coding for eCB receptors and eCB metabolic enzymes in brain tissues

Hypothalamus

Hypothalamic eCB and AEA congener levels are reported in Table 5. Diet had no significant effect on AEA, and AEA congeners, whereas 2-AG levels were increased by HFD ($p < 0.01$). Gene expression of the enzymes implicated in eCB synthesis or degradation were affected by the HFD (Fig. 4a). The expression of genes coding for ABDH4 and NAPE-PLD (*Abdh4* and *Nape-pld*, respectively), i.e., two enzymes potentially involved in AEA, OEA, and PEA biosynthesis, was significantly decreased in the HFD group compared to control rats ($p < 0.05$), and so was the mRNA coding for FAAH (*Faah*), a major enzyme for AEA, OEA, and PEA degradation ($p < 0.05$). Thus, the concurrent downregulation of biosynthetic and inactivating enzyme expression may explain the lack of effect of the HFD on levels of AEA and its congeners. Concerning 2-AG, the expression of the mRNA coding for its biosynthetic enzymes (*Magl* and *Dagl-α* or *Dagl-β*) was unchanged with diet while the mRNA coding for its degradation enzymes was either decreased (*Abdh6* gene) or increased (*Abdh12* gene) in the HFD group ($p < 0.05$). Finally, the expression of mRNAs coding for CB1 and CB2 (*Cnr1* and *Cnr2*, respectively) was not altered in the HFD group, whereas *Trpv1* mRNA was significantly decreased (Fig. 4a; $p < 0.05$).

Exercise had no significant effect on AEA, 2-AG, or AEA congener levels (Table 5) nor on the mRNA expression of genes coding for eCB receptors (CB1, CB2). *Gde-1* and *Nape-pld* mRNA expression was significantly decreased with exercise in rats on the standard diet ($p < 0.05$ for both) but not in rats on HFD (Fig. 4a). Expression of mRNA for PTNPN-22 (*Ptnpn-22*), another enzyme potentially involved in AEA, OEA, and PEA biosynthesis, was instead lowered by exercise training only in the rats fed with the HFD. Concerning 2-AG biosynthesis or degradation, only *Abdh6* mRNA levels were significantly decreased with exercise training in lean rats (Ctl + training group, $p < 0.05$).

Hippocampus

Hippocampal eCB and AEA congener levels are reported in Table 5 and results of qPCR analyses for the hippocampus are presented in Fig. 4b. The levels of eCBs and AEA congeners were not affected by diet or exercise, whereas *Cnr1* mRNA expression was significantly increased in HFD, HFD + training, and Ctl + training groups compared to the Ctl group ($p < 0.05$ for all). *Trpv1* mRNA levels were also increased in the HFD + training group compared to the Ctl group ($p < 0.05$). Concerning gene expression of eCB biosynthetic or degrading enzymes, none of them was modified by the HFD. The mRNA expression of *Gde-1* was increased and that of *Nape-pld* was decreased for Ctl + training and HFD + training groups ($p < 0.05$ for both), and *Faah* mRNA levels were increased in these two groups compared to the respective ctl groups ($p < 0.05$ for both). The mRNA expression of the 2-AG biosynthetic enzyme DAGL-α was significantly increased in the HFD + training group as was that of the 2-AG degrading enzymes ABDH6, ABDH12, and MAGL ($p < 0.05$ for all).

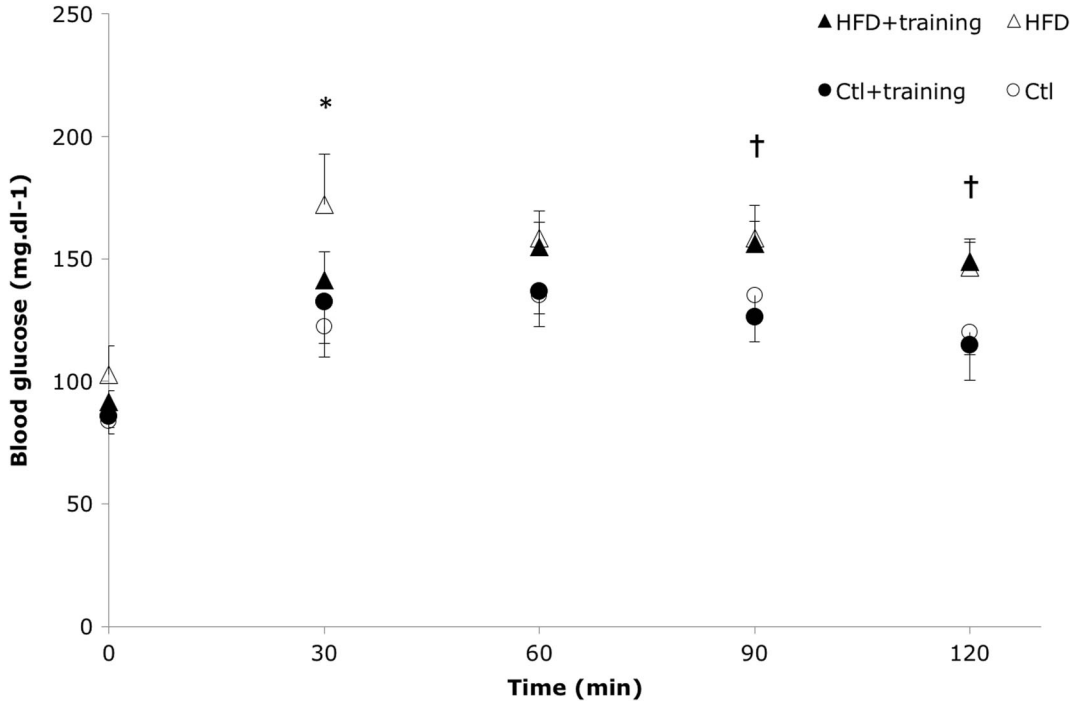

Fig. 3 Blood glucose levels at 0, 30, 60, 90, and 120 min during the oral glucose tolerance test in control (ctl), high-fat diet (HFD), control with chronic exercise (ctl + training), and high-fat diet with chronic exercise (HFD + training) groups. *Significant difference between HFD group and all other groups: $p < 0.01$. †ctl + training significantly different from HFD and HFD + training groups: $p < 0.05$

Table 5 Hypothalamic and hippocampal concentrations of endocannabinoids and anandamide congeners in control (Ctl), high-fat diet (HFD), control with chronic exercise (Ctl + training), and high-fat diet with chronic exercise (HFD + training) groups

	Ctl	HFD	Ctl + training	HFD + training	Main effects y ANOVA	F values	p
Hypothalamus							
AEA (pmol g^{-1})	27.82 ± 22.13	37.66 ± 16.61	24.54 ± 9.82	36.16 ± 14.82	Diet	$F_{(1, 26)} = 2.56$	NS
					Ex	$F_{(1, 26)} = 0.12$	NS
					Diet × Ex	$F_{(1, 24)} = 0.01$	NS
2-AG (pmol mg^{-1})	3.71 ± 1.02	4.82 ± 1.06	3.17 ± 1.14	4.62 ± 0.69	Diet	$F_{(1, 26)} = 9.24$	0.01
					Ex	$F_{(1, 26)} = 0.78$	NS
					Diet × Ex	$F_{(1, 24)} = 0.16$	NS
PEA (pmol mg^{-1})	1.98 ± 0.27	1.90 ± 0.26	1.91 ± 0.23	1.98 ± 0.38	Diet	$F_{(1, 26)} = 0.01$	NS
					Ex	$F_{(1, 26)} = 0.00$	NS
					Diet × Ex	$F_{(1, 24)} = 0.40$	NS
OEA (pmol mg^{-1})	0.56 ± 0.86	0.42 ± 0.09	0.53 ± 0.09	0.54 ± 0.11	Diet	$F_{(1, 26)} = 2.64$	NS
					Ex	$F_{(1, 26)} = 1.84$	NS
					Diet × Ex	$F_{(1, 24)} = 3.66$	NS
Hippocampus							
AEA (pmol g^{-1})	61.38 ± 24.61	64.20 ± 17.79	58.20 ± 16.44	59.78 ± 18.27	Diet	$F_{(1, 26)} = 0.07$	NS
					Ex	$F_{(1, 26)} = 0.21$	NS
					Diet × Ex	$F_{(1, 24)} = 0.01$	NS
2-AG (pmol mg^{-1})	1.68 ± 0.31	2.38 ± 1.52	2.03 ± 0.27	1.78 ± 0.40	Diet	$F_{(1, 26)} = 0.39$	NS
					Ex	$F_{(1, 26)} = 0.12$	NS
					Diet × Ex	$F_{(1, 24)} = 1.75$	NS
PEA (pmol mg^{-1})	1.00 ± 0.25	1.07 ± 0.52	0.83 ± 0.15	0.80 ± 0.21	Diet	$F_{(1, 26)} = 0.03$	NS
					Ex	$F_{(1, 26)} = 2.90$	NS
					Diet × Ex	$F_{(1, 24)} = 0.15$	NS
OEA (pmol mg^{-1})	0.33 ± 0.07	0.40 ± 0.15	0.36 ± 0.11	0.31 ± 0.06	Diet	$F_{(1, 26)} = 0.05$	NS
					Ex	$F_{(1, 26)} = 0.37$	NS
					Diet × Ex	$F_{(1, 24)} = 1.79$	NS

Data are means ± SD

The main effects from ANOVA are as follows: *Diet* diet effect, *Ex* exercise training effect, × interaction between variables, *NS* non-significant

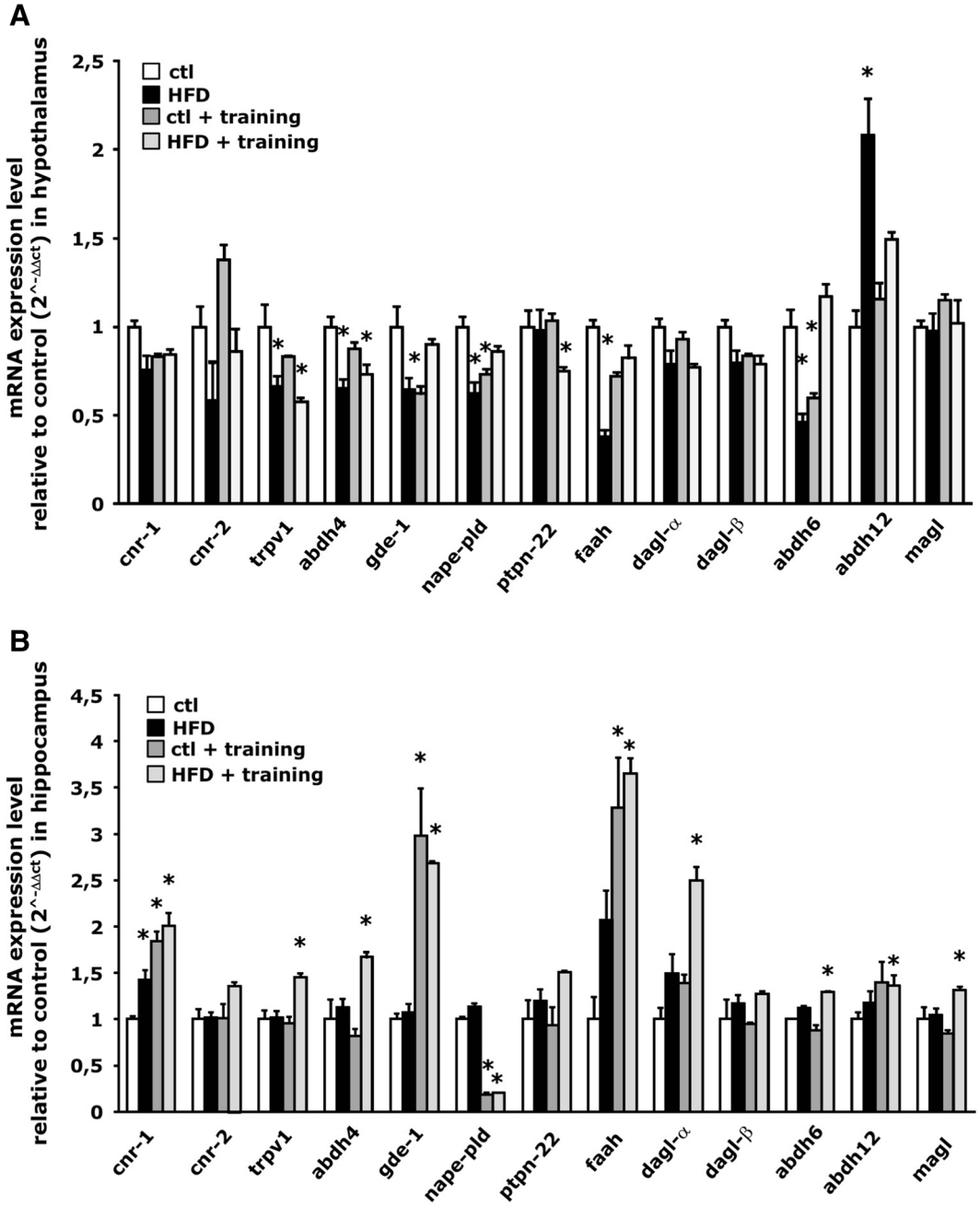

Fig. 4 Expression level analysis of the genes related to endocannabinoid metabolism and function in control (ctl), high-fat diet (HFD), control with chronic exercise (ctl + training), and high-fat diet with chronic exercise (HFD + training) groups. mRNA expression levels of genes encoding for endocannabinoid receptors (*cnr1*, *cnr2*, *trpv1*) or anabolic (*abdh4*, *gde-1*, *nape-pld*, *ptpn22*, *dagla*, *daglb*) and catabolic (*faah*, *abdh6*, *abdh12*, *magl*) enzymes were measured in the hypothalamus (**a**) and the hippocampus (**b**). The results obtained by qPCR are reported using the $2^{-\Delta\Delta ct}$ formula using hprt as housekeeping gene and data were then normalised to each corresponding control, considered with value 1. Each *column* represents the mean ± S.E.M. of at least four independent determinations performed each in quadruplicate. *Significantly different from ctl group: $p < 0.05$

Discussion

This study aimed at identifying changes in the hypothalamic and hippocampal tissue concentrations of 2-AG; AEA; and two AEA congeners, OEA and PEA, together with corresponding alterations in the expression of genes encoding for eCB receptors (CB1, CB2) and enzymes involved in the anabolic (ABHD4, GDE-1, NAPE-PLD, and PTNP-22, for AEA, OEA, and PEA; DAGL-α and DAGL-β, for 2-AG) and catabolic (FAAH, for AEA, OEA and PEA; ABHD6, ABHD12 and MAGL, for 2-AG) pathways of these mediators, after 12 weeks of

endurance training in Wistar rats fed with a standard diet or a HFD.

Previous studies in diet-induced obesity (DIO) mice or genetic models of obesity reported a dysregulation of the eCBs and particularly an increase of 2-AG in the hypothalamus [14] [4]. We confirm these results in all Wistar rats fed with the HFD in the present study. As the ECS has an important role in the regulation of food intake through hypothalamic pathways [30], this 2-AG increase might participate in the higher accumulated caloric intake observed in these rats. According to the gene expression results of biosynthetic and degrading enzymes, this 2-AG increase is difficult to explain for two main reasons. First, the HFD group presented at the same time a decrease and an increase in gene expression of two 2-AG-degrading enzymes (*Abdh6* and *Abdh12*, respectively) that are known to participate only for about 15% in 2-AG hydrolysis [5]. Secondly, these results on *Abdh6* and *Abdh12* expression were not observed in the exercise training group fed the HFD, where the same trends should have been observed to account for the similar changes in 2-AG levels. Thus, more than the mRNA expression of biosynthetic and/or degrading enzymes, enzymatic activities and eCB biosynthetic precursors might be involved in 2-AG level increase induced by HFD. Di Marzo et al. [14] indeed explain this 2-AG increase with defective leptin signaling. Leptin inhibits 2-AG biosynthesis by likely decreasing the formation of diacylglycerol (DAG) precursors [14]. Nevertheless, these results should be taken with caution as this leptin signaling decrease is not systematically observed in Wistar rats fed with high-fat diet [7], and other feeding-regulated hormones, such as grehlin and glucocorticoids, are also involved in the regulation of hypothalamic eCB levels [43]. Furthermore, in the lateral hypothalamus and arcuate nucleus (two areas of the hypothalamus) of mice fed a HFD, the increased 2-AG levels have been recently shown to be accompanied by increased DAGL-α protein expression [10] [11].

Our results confirm at the mRNA level that the expression of hypothalamic CB1 is not affected by HFD in rats. Previous rat studies have shown a lack of alteration in CB1 density with HFD [22] models. Considering that CB1 agonist levels might regulate CB1 receptor density in the hypothalamus [44], this is consistent with the fact that only the levels of 2-AG, and not also AEA, were found here to be altered in this brain area. Our results however do not exclude transient changes in CB1 or *Cnr1* expression over the course of the study. Indeed, South et al. [44] observed a transient increase in mouse hypothalamic CB1 density after 3 weeks of HFD that was normalized at the end of the 20 weeks of HFD, suggesting temporal CB1 alterations during obesity induction in rodents.

Interestingly, *Trpv1* gene expression was decreased with the HFD. To our knowledge only Baboota et al. [2] has reported a down-regulation in the expression of this gene in the mouse hypothalamus after a HFD period. The down-regulation of *Trpv1*, observed now here also in rats, may play a role in the hyperglycemia observed in the HFD group. Indeed, Zsombok [52] suggested that TRPV1 activation in the paraventricular nucleus of the hypothalamus could lower blood glucose.

Unlike the hypothalamus, the HFD did not alter 2-AG levels in the hippocampus in our study, nor did it alter the levels of AEA and its congeners. However, the ECS in this brain area was previously shown to be sensitive to this kind of diet [34, 41], with increased AEA and 2-AG hippocampal concentrations in mice after a 12-week HFD [34], and Rivera et al. [41] reporting increased AEA, and other acylethanolamide (OEA, PEA) but not 2-AG, levels also in rats with 12-week HFD. While these previous studies reported an unbalanced ratio between the expression of eCB biosynthesis and degradation enzymes, we did not observe any significant change in the levels of the mRNAs coding for these enzymes, which may explain the absence of eCB level alterations. Differences in the diet composition between studies may partly explain these discrepancies. Different amounts of polyunsaturated fatty acid precursors in the diet are for example known to modulate the availability of eCB biosynthetic precursors [1]. Nevertheless, as shown in a previous study [34], where the mRNA coding for CB1 (*Cnr1*) and the CB1 protein were overexpressed, the hippocampal ECS may be altered following a HFD and contribute to the development of obesity or related metabolic alterations. Accordingly, an increase in CB1 expression was shown to participate in the regulation of neuroplasticity involved in the hedonic aspect of eating [34]. Here, we found that rats fed with HFD showed increased *Cnr1* mRNA expression as compared to rats fed with standard diet, which correlates with a higher accumulated caloric intake in HFD rats.

The up-regulation of hippocampal *Cnr1* expression was also found with exercise training combined with HFD. Chronic exercise did not appear to counteract ECS overactivation and, in fact, seems even to induce this effect independently of the diet. Indeed, *Cnr1* mRNA and the majority of the genes coding for biosynthetic and degrading eCB enzymes were increased in Ctl + training rats, suggesting an increase in eCB turnover and signaling. This result is not surprising as Hill et al. [25] have already observed an increase in AEA levels and in CB1 agonist binding density after 8 days of free access to a running wheel in the hippocampus of lean Wistar rats. They demonstrated that this ECS overactivation in this brain area was required for chronic exercise-induced neuroplasticity. However, exercise does not produce the reversal of CB1 overactivation induced by HFD in spite of the slowing down of calorie intake accumulation observed with exercise training. Studies of different hippocampal regions will be needed to determine whether exercise and HFD

selectively affect eCB signaling in the different subpopulations of neurons in this brain area.

Altogether, these data suggest that exercise training induces hippocampal CB1 signaling, which may be potentially involved in enhanced neuroplasticity, thus possibly explaining the improved cognitive performance and mood observed typically with exercise [47].

In contrast to the hippocampus, the hypothalamic ECS seems to be less sensitive to exercise, as no change in CB1 and CB2 were observed in HFD and Ctl rats, whether they exercised or not. These results may differentiate chronic exercise from chronic stress, considering that repeated stress exposure leads to ECS alterations in the hypothalamus and other brain area involved in the stress response [42]. Wamsteeker et al. [50] showed a functional downregulation of CB1 receptor in the paraventricular nucleus of the hypothalamus after repetitive immobilization stress in adolescent Sprague Dawley rats. Indeed, exercise differs from other stressors. It activates a number of systems related to the stress response but other mechanisms associated with chronic exercise exist to reduce the negative effect of this stressor [46], thus possibly avoiding endocannabinoid-signaling impairment.

Our study suffers from several limitations. First, gene expression quantification without data on receptor or enzyme protein levels or function limits the interpretation of our results. Secondly, the brain areas studied were limited to the hippocampus and hypothalamus. Other brain structures involved in the control of eating and voluntary exercise behaviors, such as the ventral tegmental area and striatum, are highly sensitive to eCB signaling [13, 20, 44]. Measurements of ECS activities in these areas would have allowed to provide a global view of the different structures affected by exercise and food intake. Third, treadmill was chosen instead of running wheel as exercise protocol. Even though both of these protocols are known to induce neural plasticity [33], the forced exercise could induce additional stress compared to the voluntary exercise [19] and thus affect the ECS differently. However, our results (i.e., *Cnr1* upregulation in the hippocampus) do not seem to be the mere consequence of chronic stress induced by forced exercise. In fact, Hill et al. [24] reported hippocampal CB1 down-regulation following 21 days of chronic stress.

In summary, we have confirmed here that HFDs are accompanied by changes in the expression of genes encoding for eCB receptors and enzymes involved in the anabolic and catabolic pathways of the ECS in the hippocampus. These changes, and particularly the alteration of hypothlamic 2-AG levels and hippocampal CB1 receptor gene expression, may participate in weight gain and glucose metabolic perturbation observed with the HFD. While chronic exercise improves weight alterations and avoids hyperglycemia induced by HFD, the alterations in ECS gene expression occurring during the latter diet are not reversed, and chronic exercise may even result in several hippocampal ECS responses similar to those observed with HFD. These results highlight the need for additional investigations about the role of the ECS in the beneficial brain adaptations induced by chronic exercise.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Artmann A, Petersen G, Hellgren LI, Boberg J, Skonberg C, Nellemann C, Hansen SH, Hansen HS (2008) Influence of dietary fatty acids on endocannabinoid and N-acylethanolamine levels in rat brain, liver and small intestine. Biochim Biophys Acta 1781: 200–212
2. Baboota RK, Murtaza N, Jagtap S, Singh DP, Karmase A, Kaur J, Bhutani KK, Boparai RK, Premkumar LS, Kondepudi KK et al (2014) Capsaicin-induced transcriptional changes in hypothalamus and alterations in gut microbial count in high fat diet fed mice. J Nutr Biochem 25:893–902
3. Bennetzen MF (2011) Investigations of the endocannabinoid system in adipose tissue: effects of obesity/weight loss and treatment options. Dan Med Bull 58:B4269
4. Bisogno T, Mahadevan A, Coccurello R, Chang JW, Allara M, Chen Y, Giacovazzo G, Lichtman A, Cravatt B, Moles A et al (2013) A novel fluorophosphonate inhibitor of the biosynthesis of the endocannabinoid 2-arachidonoylglycerol with potential anti-obesity effects. Br J Pharmacol 169:784–793
5. Blankman JL, Simon GM, Cravatt BF (2007) A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. Chem Biol 14:1347–1356
6. Cadas H, Gaillet S, Beltramo M, Venance L, Piomelli D (1996) Biosynthesis of an endogenous cannabinoid precursor in neurons and its control by calcium and cAMP. J Neurosci 16:3934–3942
7. Chalkley SM, Hettiarachchi M, Chisholm DJ, Kraegen EW (2002) Long-term high-fat feeding leads to severe insulin resistance but not diabetes in Wistar rats. Am J Physiol Endocrinol Metab 282: E1231–E1238
8. Christensen R, Kristensen PK, Bartels EM, Bliddal H, Astrup A (2007) Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. Lancet 370:1706–1713
9. Cota D, Marsicano G, Tschop M, Grubler Y, Flachskamm C, Schubert M, Auer D, Yassouridis A, Thone-Reineke C, Ortmann S et al (2003) The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. J Clin Invest 112:423–431
10. Cristino L, Busetto G, Imperatore R, Ferrandino I, Palomba L, Silvestri C, Petrosino S, Orlando P, Bentivoglio M, Mackie K et al (2013) Obesity-driven synaptic remodeling affects endocannabinoid control of orexinergic neurons. Proc Natl Acad Sci U S A 110:E2229–E2238
11. Cristino L, Luongo L, Imperatore R, Boccella S, Becker T, Morello G, Piscitelli F, Busetto G, Maione S, Di Marzo V (2016) Orexin-a and endocannabinoid activation of the descending antinociceptive

pathway underlies altered pain perception in leptin signaling deficiency. Neuropsychopharmacology 41:508–520
12. D'Eon TM, Pierce KA, Roix JJ, Tyler A, Chen H, Teixeira SR (2008) The role of adipocyte insulin resistance in the pathogenesis of obesity-related elevations in endocannabinoids. Diabetes 57: 1262–1268
13. De Chiara V, Errico F, Musella A, Rossi S, Mataluni G, Sacchetti L, Siracusano A, Castelli M, Cavasinni F, Bernardi G et al Voluntary exercise and sucrose consumption enhance cannabinoid CB1 receptor sensitivity in the striatum. Neuropsychopharmacology 35:374–387
14. Di Marzo V, Goparaju SK, Wang L, Liu J, Batkai S, Jarai Z, Fezza F, Miura GI, Palmiter RD, Sugiura T et al (2001) Leptin-regulated endocannabinoids are involved in maintaining food intake. Nature 410:822–825
15. Di Marzo V, Matias I (2005) Endocannabinoid control of food intake and energy balance. Nat Neurosci 8:585–589
16. Di Marzo V (2008) Endocannabinoids: synthesis and degradation. Rev Physiol Biochem Pharmacol 160:1–24
17. Di Marzo V, Cote M, Matias I, Lemieux I, Arsenault BJ, Cartier A, Piscitelli F, Petrosino S, Almeras N, Despres JP (2009a) Changes in plasma endocannabinoid levels in viscerally obese men following a 1 year lifestyle modification programme and waist circumference reduction: associations with changes in metabolic risk factors. Diabetologia 52:213–217
18. Di Marzo V, Verrijken A, Hakkarainen A, Petrosino S, Mertens I, Lundbom N, Piscitelli F, Westerbacka J, Soro-Paavonen A, Matias I et al (2009b) Role of insulin as a negative regulator of plasma endocannabinoid levels in obese and nonobese subjects. Eur J Endocrinol 161:715–722
19. Dishman RK (1997) Brain monoamines, exercise, and behavioral stress: animal models. Med Sci Sports Exerc 29:63–74
20. Dubreucq S, Durand A, Matias I, Benard G, Richard E, Soria-Gomez E, Glangetas C, Groc L, Wadleigh A, Massa F et al (2013) Ventral tegmental area cannabinoid type-1 receptors control voluntary exercise performance. Biol Psychiatry 73:895–903
21. Gamelin FX, Aucouturier J, Iannotti FA, Piscitelli F, Mazzarella E, Aveta T, Leriche M, Dupont E, Cieniewski-Bernard C, Montel V, et al. (2016) Effects of chronic exercise on the endocannabinoid system in Wistar rats with high-fat diet-induced obesity. J Physiol Biochem
22. Harrold JA, Elliott JC, King PJ, Widdowson PS, Williams G (2002) Down-regulation of cannabinoid-1 (CB-1) receptors in specific extrahypothalamic regions of rats with dietary obesity: a role for endogenous cannabinoids in driving appetite for palatable food? Brain Res 952:232–238
23. Heyman E, Gamelin FX, Goekint M, Piscitelli F, Roelands B, Leclair E, Di Marzo V, Meeusen R (2012) Intense exercise increases circulating endocannabinoid and BDNF levels in humans—possible implications for reward and depression. Psychoneuroendocrinology 37:844–851
24. Hill MN, Patel S, Carrier EJ, Rademacher DJ, Ormerod BK, Hillard CJ, Gorzalka BB (2005) Downregulation of endocannabinoid signaling in the hippocampus following chronic unpredictable stress. Neuropsychopharmacology 30:508–515
25. Hill MN, Titterness AK, Morrish AC, Carrier EJ, Lee TT, Gil-Mohapel J, Gorzalka BB, Hillard CJ, Christie BR (2010) Endogenous cannabinoid signaling is required for voluntary exercise-induced enhancement of progenitor cell proliferation in the hippocampus. Hippocampus 20:513–523
26. Iannotti FA, Piscitelli F, Martella A, Mazzarella E, Allara M, Palmieri V, Parrella C, Capasso R, Di Marzo V (2013) Analysis of the "endocannabinoidome" in peripheral tissues of obese Zucker rats. Prostaglandins Leukot Essent Fatty Acids 89:127–135
27. Jamshidi N, Taylor DA (2001) Anandamide administration into the ventromedial hypothalamus stimulates appetite in rats. Br J Pharmacol 134:1151–1154
28. Jo YH, Chen YJ, Chua SC Jr, Talmage DA, Role LW (2005) Integration of endocannabinoid and leptin signaling in an appetite-related neural circuit. Neuron 48:1055–1066
29. Kim KW, Zhao L, Donato J Jr, Kohno D, Xu Y, Elias CF, Lee C, Parker KL, Elmquist JK (2011) Steroidogenic factor 1 directs programs regulating diet-induced thermogenesis and leptin action in the ventral medial hypothalamic nucleus. Proc Natl Acad Sci U S A 108:10673–10678
30. Kirkham TC, Williams CM, Fezza F, Di Marzo V (2002) Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. Br J Pharmacol 136:550–557
31. Kirkham TC (2009) Endocannabinoids and the non-homeostatic control of appetite. Curr Top Behav Neurosci 1:231–253
32. Krawczewski Carhuatanta KA, Demuro G, Tschop MH, Pfluger PT, Benoit SC, Obici S (2011) Voluntary exercise improves high-fat diet-induced leptin resistance independent of adiposity. Endocrinology 152:2655–2664
33. Liu YF, Chen HI, Wu CL, Kuo YM, Yu L, Huang AM, Wu FS, Chuang JI, Jen CJ (2009) Differential effects of treadmill running and wheel running on spatial or aversive learning and memory: roles of amygdalar brain-derived neurotrophic factor and synaptotagmin I. J Physiol 587:3221–3231
34. Massa F, Mancini G, Schmidt H, Steindel F, Mackie K, Angioni C, Oliet SH, Geisslinger G, Lutz B (2010) Alterations in the hippocampal endocannabinoid system in diet-induced obese mice. J Neurosci 30:6273–6281
35. Matias I, Petrosino S, Racioppi A, Capasso R, Izzo AA, Di Marzo V (2008) Dysregulation of peripheral endocannabinoid levels in hyperglycemia and obesity: effect of high fat diets. Mol Cell Endocrinol 286:S66–S78
36. Pagotto U, Marsicano G, Cota D, Lutz B, Pasquali R (2006) The emerging role of the endocannabinoid system in endocrine regulation and energy balance. Endocr Rev 27:73–100
37. Pedersen BK, Saltin B (2006) Evidence for prescribing exercise as therapy in chronic disease. Scand J Med Sci Sports 16(Suppl 1):3–63
38. Pi-Sunyer FX, Aronne LJ, Heshmati HM, Devin J, Rosenstock J & Group RI-NAS (2006) Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients: RIO-North America: a randomized controlled trial. JAMA 295:761–775
39. Purves RD (1992) Optimum numerical integration methods for estimation of area-under-the-curve (AUC) and area-under-the-moment-curve (AUMC). J Pharmacokinet Biopharm 20:211–226
40. Raichlen DA, Foster AD, Gerdeman GL, Seillier A, Giuffrida A (2012) Wired to run: exercise-induced endocannabinoid signaling in humans and cursorial mammals with implications for the 'runner's high. J Exp Biol 215:1331–1336
41. Rivera P, Luque-Rojas MJ, Pastor A, Blanco E, Pavon FJ, Serrano A, Crespillo A, Vida M, Grondona JM, Cifuentes M et al (2013) Diet-dependent modulation of hippocampal expression of endocannabinoid signaling-related proteins in cannabinoid antagonist-treated obese rats. Eur J Neurosci 37:105–117
42. Senst L, Bains J (2014) Neuromodulators, stress and plasticity: a role for endocannabinoid signalling. J Exp Biol 217:102–108
43. Silvestri C, Di Marzo V (2013) The endocannabinoid system in energy homeostasis and the etiopathology of metabolic disorders. Cell Metab 17:475–490
44. South T, Huang XF (2008) Temporal and site-specific brain alterations in CB1 receptor binding in high fat diet-induced obesity in C57Bl/6 mice. J Neuroendocrinol 20:1288–1294

45. Sparling PB, Giuffrida A, Piomelli D, Rosskopf L, Dietrich A (2003) Exercise activates the endocannabinoid system. Neuroreport 14:2209–2211
46. Stranahan AM, Lee K, Mattson MP (2008) Central mechanisms of HPA axis regulation by voluntary exercise. NeuroMolecular Med 10:118–127
47. Tantimonaco M, Ceci R, Sabatini S, Catani MV, Rossi A, Gasperi V, Maccarrone M (2014) Physical activity and the endocannabinoid system: an overview. Cell Mol Life Sci 71:2681–2698
48. van Praag H, Shubert T, Zhao C, Gage FH (2005) Exercise enhances learning and hippocampal neurogenesis in aged mice. J Neurosci 25:8680–8685
49. Verty AN, McGregor IS, Mallet PE (2004) The dopamine receptor antagonist SCH 23390 attenuates feeding induced by Delta9-tetrahydrocannabinol. Brain Res 1020:188–195
50. Wamsteeker JI, Kuzmiski JB, Bains JS (2010) Repeated stress impairs endocannabinoid signaling in the paraventricular nucleus of the hypothalamus. J Neurosci 30:11188–11196
51. Yan ZC, Liu DY, Zhang LL, Shen CY, Ma QL, Cao TB, Wang LJ, Nie H, Zidek W, Tepel M et al (2007) Exercise reduces adipose tissue via cannabinoid receptor type 1 which is regulated by peroxisome proliferator-activated receptor-delta. Biochem Biophys Res Commun 354:427–433
52. Zsombok A (2013) Vanilloid receptors—do they have a role in whole body metabolism? Evidence from TRPV1. J Diabetes Complicat 27:287–292