Increased Anandamide and Decreased Pain and Depression after Exercise in Fibromyalgia

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ABSTRACT

STENSSON, N., B. GERDLE, M. ERNBERG, K. MANNERKORPI, E. KOSEK, and B. GHAFOURI. Increased Anandamide and Decreased Pain and Depression after Exercise in Fibromyalgia. *Med. Sci. Sports Exerc.*, Vol. 52, No. 7, pp. 1617–1628, 2020. **Purpose:** Physical exercise is increasingly being promoted by health care for chronic pain conditions with beneficial outcomes, such as pain and fatigue reduction, and increased quality of life. Nevertheless, knowledge about biochemical consequences of physical exercise in chronic pain is still relatively poor. The endocannabinoid system has been suggested to play a role for acute exercise-induced reward and pain inhibition. The aim of this study is to investigate the chronic outcomes of resistance exercise on levels of endocannabinoids and related lipids in fibromyalgia (FM). **Methods:** This study examine the outcomes of a 15-wk person-centered resistance exercise program on plasma levels of the lipid mediators; anandamide, 2-arachidonoylglycerol (2-AG), oleoylethanolamide, palmitoylethanolamide, and stearoylethanolamide (SEA) sampled from 37 women with FM and 33 healthy controls. The associations between clinical scorings of pain, depression, anxiety, fatigue, and muscle strength with levels of these lipid mediators before and after the exercise program are also analyzed. **Results:** After the 15-wk exercise program, anandamide levels were significantly increased, and SEA levels significantly decreased in FM. Pain intensity and depression scorings decreased and muscle strength increased, and in a multivariate context, muscle strength was positively associated with 2-AG levels after the resistance exercise program in FM. **Conclusions:** The increased anandamide and decreased SEA in women with FM after the 15-wk program might point to a chronic effect of resistance exercise. Pain and depression scorings decreased in the FM group after the program, but no associations between pain, depression, and lipid level changes were assured. **Key Words:** FIBROMYALGIA, CHRONIC PAIN, PHYSICAL EXERCISE, ANANDAMIDE, ENDOCANNABINOIDS

In a historical perspective, people with chronic pain usually were told to rest. However, general advice now is to keep active, and available evidence suggests that interventions involving physical activity and exercise may reduce pain severity and increase physical function and quality of life (1).

Manifested as chronic widespread musculoskeletal pain (CWP) and generalized hyperalgesia, fibromyalgia (FM) is a heterogeneous condition with a prevalence of 2% to 4% afflicting women to a higher degree than men (2). Other common symptoms of FM include depression, anxiety, stiffness, fatigue, sleep disturbances, and cognitive dysfunction (3). Prevalent consequences of FM are reduced quality of life, increased use of health resources, and loss of work productivity.

Current evidence demonstrates that strength training may be as beneficial as aerobic exercise in FM, with outcomes as reduction in pain, fatigue, number of tender points, depression, and anxiety, as well as with increased functional capacity and quality of life (4).

The endocannabinoid system (EC) is associated with multiple biochemical actions by modulating, for example, not only pain (5) and inflammation (6), but also emotions, anxiety, and stress (7). Moreover, this system has been reported to be acutely activated by physical activity (8).

The lipid mediators of the EC are known as the endocannabinoids (eCB) where arachidonoylethanolamide (AEA) (also named anandamide) and 2-arachidonoylglycerol

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(2-AG) are well characterized as activators of the cannabinoid receptors (CB_1 and CB_2). Other ethanolamide lipid mediators, namely, the *N*-acylethanolamines (NAE), do not activate CB receptors, but partially share metabolic enzymes with eCB and have other molecular targets. Both palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) can activate peroxisome proliferator activating receptor-α (PPAR-α) (9,10), and stearoylethanolamide (SEA) has been proposed to activate PPAR-γ (11).

Emerging data suggest that the EC—along with other endogenous systems—play an important role for acute exercise-induced hypoalgesia (EIH), which in a recent review from Rice et al (12) is suggested to be impaired in some people with chronic pain.

Basal differences of levels of eCB and NAE have been reported between FM and healthy control (HC). Increased plasma anandamide was reported in FM compared with controls (13), we have reported increased plasma OEA, PEA, SEA, and 2-AG levels in FM compared with HC (14). Furthermore, both acute aerobic and strength exercise have been demonstrated to affect blood levels of eCB and NAE (15). Concerning aerobic exercise, plasma anandamide levels were significantly increased in humans after 30-min running (16), and after a 60-min bicycling (17). Concerning strength exercise, plasma anandamide, OEA, PEA, and 2-AG increased after 7.5-min resistance exercise and produced antinociception in rats (18), and 3-min submaximal isometric exercise produced EIH and increased plasma levels of anandamide, OEA, PEA, DHEA, 2-AG, and 2-OG in humans (19).

We have previously reported altered levels of eCB/NAE in response to physical activity in CWP (20). In that report trapezius muscle PEA and SEA levels decreased in response to an acute—20-min repetitive low-force—exercise in women with CWP, but not in controls (20). To the best of our knowledge, that is the only study that investigated local response of acute muscle work on NAE in FM/CWP. The main focus in the present study is to investigate chronic effects of physical resistance exercise on plasma levels of eCB/NAE in FM, which was done by measuring levels of anandamide, OEA, PEA, SEA, and 2-AG in FM and HC before and after a 15-wk resistance exercise program. Further, to investigate if the chronic exercise affects the response of the acute exercise in muscle, we analyzed levels of OEA, PEA, and SEA in microdialysate sampled from vastus lateralis muscles before and after a 20-min brief standardized work of repeated dynamic knee extensions.

We hypothesize that EIH could be impaired in FM and that the EC could play a role in this defect. However, there are no reports about the effects of chronic physical exercise on eCB/NAE in FM in the literature. The aims in this study are, therefore, to investigate if chronic resistance exercise affect plasma levels of eCB/NAE in subjects with FM and in HC and to analyze associations between levels of these lipid mediators and pain characteristics, psychological aspects, health status, and muscle strength.

PARTICIPANTS AND METHODS

This study is a nonrandomized substudy of a randomized controlled multicenter trial investigating the effects of progressive resistance exercise in women with FM (Clinicaltrials.gov NCT01226784) conducted at three sites (Gothenburg, Linköping, and Stockholm) in Sweden. Some data (pain characteristics, psychological aspects, health status, and muscle strength) have essentially been presented elsewhere (21,22), however, not on the exact same number of participants. In this study, levels of eCB and NAE have been measured and related to this earlier presented data which is the unique scope of the present work.

Participants. The recruitment process has previously been described in detail (21). The recruitment started in 2010 and data collection was completed in 2013. During the same period as the participants with FM were recruited, age-matched women were recruited as HC. For both groups, inclusion criteria included working age (20–65 yr). For the women with FM, inclusion criteria included diagnosis of FM according to the ACR-1990 classification criteria (23). The inclusion criteria for HC included no current pain. For the women with FM, exclusion criteria were high blood pressure (>160/90 mm Hg), osteoarthritis in the hip or knee, other severe somatic or psychiatric disorders, primary causes of pain other than FM, high consumption of alcohol (Audit >6), participation in a rehabilitation program within the past year, regular resistance exercise or relaxation therapy twice a week or more, inability to understand or speak Swedish, and not being able to refrain from analgesics, nonsteroidal antiinflammatory drugs, or hypnotics for the 48 h before examinations (1 wk for nonsteroidal antiinflammatory drugs in the MD experiments). In this substudy, data from two partially overlapping cohorts were analyzed. In the primary cohort, plasma from 37 participants with FM and 33 HC were analyzed pre and post a 15-wk progressive resistance exercise program. In the secondary cohort, MD data from 24 FM and 27 HC were analyzed.

The usual physical activity level of the participants in the primary cohort was measured using Leisure Time Physical Activity Instrument. With this instrument, participants estimate light, moderate, and vigorous physical activity per week, during the last 4 wk.

The number of subjects needed in order to achieve sufficient power in this substudy was based on glutamate concentrations from subjects in the present cohort previously reported in the study of Gerdle et al(24). For between group differences, a mean difference with a standard deviation of 30 μmol L^{-1} was assumed. Expectation of a mean difference of 25 μmol L^{-1}, required a sample size of 24 subjects in each group to reject the null hypothesis with a power of 0.80 and a probability of <0.05 (two-tailed). For the estimation of sample size for analyzing changes within groups we assumed that the mean difference should have a standard deviation of 25 μmol L^{-1}. Expectation of a mean change of 15 μmol L^{-1}, required a sample size of 24 pairs of subjects to reject the null hypothesis with a power of 0.80 and a probability of <0.05 (two tailed). Hence, this power analysis can be considered as

a *post hoc* calculation assuring that the number of subjects in the present study has the power to reveal relevant differences. As this calculation were based on glutamate concentrations and not eCB and NAE, *post hoc* power calculations were performed for between and within group difference for each lipid using probability of 0.05 (two tailed), which is presented in the result section. The sample size and power calculations were made using the computer program Power and Sample Size Calculations (v. 3.0.43,http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize).

The study was conducted in accordance with the Helsinki Declaration and Good Clinical Practice. The Central Ethical Review Board in Stockholm approved the study (Dnr: 2010/1121–31/3). All participants received verbal and written information about the study and gave their written consent. The participants were compensated economically for their participation.

The resistance exercise intervention. The intervention has earlier been described in detail (21). Briefly, the resistance exercise program was performed twice a week for 15 wk and was supervised by experienced physiotherapists, and was performed in a person-centered manner. It was conducted at physiotherapy premises and at a local gym at four different sites in groups comprising five to seven participants to promote interaction between participants and to facilitate physiotherapeutic guidance. An individual introductory meeting was conducted before the start of the intervention. The introductory meeting included exercise instructions, testing of 1-min repetition maximum (1 RM) and adjustment of loads and modifications of specific exercises according to individual conditions and according to self-efficacy principles of each participant's confidence in their ability to perform each exercise and to manage specific loads. The meeting resulted in a written protocol with descriptions of specific exercises and loads, which was used by each participant as an exercise program at each exercise session. The program comprised large muscle groups for lower and upper extremities, activating hip extensor, hip flexors, knee extensors, knee flexors, calf muscles, elbow-flexors, and muscles stabilizing the core.

Exercise load during weeks 1 to 2 was 40% of 1RM with 15 to 20 repetitions in one to two sets. During weeks 3 to 5, it was increased to 60% of 1RM with 10 to 12 repetitions in 1–2 sets, and during weeks 6 to 15 to 80% of 1RM with five to eight repetitions in one to two sets. Each session comprised 10 min warm-up and 50 min of standardized resistance exercise.

Clinical parameters. This section describes the subjective and semiobjective measures of pain, general health status anxiety, depression, anxiety, fatigue, and physical capacity and will be referred as "clinical parameters" in the following sections.

Pain assessments. The subjects assessed their whole-body pain intensity by placing a mark on a 100-mm visual analog scale (VAS) (with the endpoints: 0 = no pain and 100 = worst possible pain; without any marks between these endpoints).

Pain sensitivity was assessed with pressure pain thresholds (PPT) using an electronic pressure algometer (Somedic, Hörby, Sweden) as previously described (24). The probe area was 1 cm^2, and pressure was applied perpendicularly to the skin at a speed corresponding to approximately 50 $kPa \cdot s^{-1}$. The participants were instructed to mark the PPT by pressing a button as the sensation of "pressure" changed to "pain." When the button was pressed or when the maximum pressure of 1500 kPa was reached, the application of pressure ceased. Algometry was performed bilaterally over the supraspinatus muscle (at origins above the scapula spine near the medial border), the lateral epicondyle (2-cm distal to the epicondyles), over the gluteus maximus (in upper outer quadrants of buttocks in anterior fold of muscle), and inside the knee (at the medial fat pad proximal to the joint line). That is, the algometry was used on 8 of the 18 tender points defined by the ACR criteria (23). A mean value of all eight anatomical sites for each subject was used for further analysis. Before the actual testing of PPT, the subjects were given instructions and were familiarized with the testing procedure.

General health status. The Fibromyalgia Impact Questionnaire (FIQ) was used to measure health status. FIQ is a disease-specific self-reported questionnaire that comprises 10 subscales of disabilities and symptoms and is scored on a scale ranging from 0 to 100. The total score is the mean of 10 subscales. A higher score indicates a worse health status.

Assessments of anxiety and depression. The Hospital Anxiety and Depression Scale (HADS), a short self-assessment questionnaire that measures symptoms of anxiety and depression, comprises seven items in each of the depression (HAD-D) and anxiety (HAD-A) scales. Possible subscale scores range from 0 to 21, with the lower score indicating the least depression and anxiety possible. A score of 7 or less indicates a noncase, a score of 8 to 10 indicates a doubtful case, and a score of 11 or more indicates a definite case.

Fatigue assessment. The multidimensional fatigue inventory (MFI)-20 comprises 20 statements on a five-point Likert scale that addresses aspects of fatigue experienced during the most recent days. The scores generate five subscales of fatigue: general fatigue, physical fatigue, mental fatigue, reduced motivation, and reduced activity. The scores range from 4 to 20 for each subscale and higher scores refer to a higher degree of fatigue. The MFI-20 has been shown to have satisfactory convergent construct validity and test–retest reliability in FM. In this study, the total scores from the five subscales were used for further analysis.

Physical capacity measures. Isometric knee-extension force (N) was measured with a dynamometer (Steve Strong®; Stig Starke HBI, Gothenburg, Sweden) using a standard protocol. The participant was in a fixed seated position with back support, knee and hip in 90° of flexion, and legs hanging freely. A nonelastic strap was placed around the ankle and attached to a pressure transducer with an amplifier. The subjects were instructed and verbally encouraged to pull the ankle strap

with maximal force for 5 s. Three trials were performed for each test, and there was a 1-min rest between each trial. The best performance out of three trials was recorded. A mean value from the right and left leg was calculated. Maximal isometric elbow-flexion force (N) in both arms, assessed one by one, was measured using a dynamometer (Isobex®; Medical Device Solutions AG, Oberburg, Switzerland). The participant was sitting without back support with the upper arm aligned with the trunk. The elbow was placed in 90° of flexion, forearm supinated. The maximum strength performed during a period of 5 s was recorded. Three trials were performed for each arm, and between each trial, there was a 1-min rest interval. The best performance out of the three trials was recorded. A mean value from the right and left arms was calculated. Hand grip force (N) bilaterally registered using Grippit® (AB Detector, Gothenburg, Sweden). The mean force over a set period (10 s) was recorded. Two trials were performed for each test, and there was a 1-min rest between each trial. The best performance of the two trials was recorded. A mean value of the right and left hands was calculated and used in the present study.

Sampling of blood and microdialysate. This description is valid for blood and muscle dialysate sampling in all subjects (FM and HC). Samplings were conducted at three different sites (Stockholm, Gothenburg, Linkoping both at A.M. and P.M. However, it was important that samples collected at P.M. (or A.M.) before the exercise program were collected also at P.M. (or A.M.) after the program to minimize the effects of daily variation of the lipids. Samples were collected before (within 1–7 d) the start of the 15-wk resistance exercise interventions and after the intervention (within 1–7 d).

Blood sampling. The venous blood samples were collected with a Vacutainer (BD Vacutainer1Eclipse™ Blood Collection Needle; BD Diagnostics, Becton, Dickinson and Company, Franklin Lakes, NJ) in a 10-mL plasma tube (BD Vacutainer1Plus Plastic K2EDTA Tubes; BD Diagnostics, Becton, Dickinson and Company). The blood samples were centrifuged for 30 min (1500*g*, RT) immediately after the collection, and the plasma was pipetted to Eppendorf tubes, aliquoted, and stored at −70°C.

Microdialysate sampling from vastus lateralis during a 20-min standardized repeated muscle work. The microdialysate (MD) sampling has essentially been described before (25). Briefly, 5 min before the insertion of MD probes into the belly of the vastus lateralis, at half the distance between the trochanter and the knee, the skin overlying was anesthetized with local anesthesia (Xylocain®, lidocaine 10 $mg \cdot mL^{-1}$; AstraZeneca, Södertälje, Sweden). The CMA 60 catheter (M Dialysis AB, Solna, Sweden) with 20 kDa cutoff 30-mm length, 0.5-mm diameter membrane was inserted into the most painful vastus muscle. The set perfusion rate was 5 $mL \cdot min^{-1}$ and dialysate was collected in 20-min intervals. After the 2 h of stabilization, that is, the trauma phase, baseline dialysates were sampled between 120 and 140 min. This was followed by sampling of dialysates during the 20 min of standardized repeated dynamic contractions of the quadriceps muscle (brief work) and finally during the 60-min recovery period. The brief task was to extend the knee 15° by slowly lifting the leg (resting with the knee bent 15° on a Pilatus ball; 55 cm diameter) to a straight position (0°) and then slowly lowering it down on the ball for participants with a current pain intensity in the exercising leg greater than 40 on a 0–100 VAS. For the other participants (current pain intensity less than 40 on a 0–100 VAS) the knee extension was 20°. Finally, seven subjects in FM worked with the 15° angle and the remaining 17 with 20°. All HC were performed with the 20° angle. Each repetition lasted for 5 s, without any rest between repetitions.

All samples were kept on ice during the experiment, and after the experiment, the dialysate was stored in aliquots at −70°C until day of analysis. When the MD sampling was finished, the catheter was removed, and the membrane was checked to make sure that no damage had occurred.

Analysis of lipid concentrations. The lipids were extracted, and the concentrations were analyzed using a liquid chromatography tandem mass spectrometry (LC-MS/MS) method based on a previously published method (14). Before the measurements 300 μL of plasma were thawed and vortexed, and 30 μL of a mixture containing a deuterated internal standard (anadamide-d4, OEA-d4, PEA-d4, and SEA-d3 (50 nM)) and 2AG-d5 (1000 nM) were added to each plasma sample. The samples were the extracted using Octyl SPE columns (6 mL, 200 mg) (Biotage, Uppsala, Sweden) as in (14).

Regarding the MD samples, briefly, 50-μL microdialysate was thawed and vortexed, and 30 μL of a mixture containing the deuterated internal standard (OEA-d4, PEA-d4, and SEA-d3 [25 nM]) were added to each sample. Samples were then prepared following the protocol described in the study of Stensson et al (26).

On the day of analysis, samples were reconstituted in 30 μL of LC mobile phase A. The injection volume was 10 μL. All standards and internal standards were purchased from Cayman Chemicals (Ann Arbor, MI). We used a high pressure liquid chromatography coupled to tandem mass spectrometry system containing a Thermo Scientific Accela AS auto sampler and Accela 1250 pump coupled to a Thermo Scientific TSQ Quantum Access max triple quadrupole mass spectrometer with a HESI II probe as ionization source. LC was performed using gradient elution with mobile phase A, containing methanol-milliQ water-acetonitrile (4/4/2) (v/v/v) and mobile phase B containing methanol-ACN (7/3) (v/v) with 0.1% (v/v) formic acid and 1 $g \cdot L^{-1}$ ammonium acetate in A and B. Gradient elution was applied with a constant flow of 250 $\mu L \cdot min^{-1}$ and started with 100% A during the first 1.5 min and followed this using a linear increase toward 100% B, which was achieved after 9 min in total. Between the 11th and 12th minutes, the gradient changed linear to 100% A, which was maintained for 1 min. An Xbridge C8 analytical column (2.1 mm × 150 mm) with the particle size 2.5 μm obtained from Waters (Dublin, Ireland). We used the following selected reaction monitoring (m/z) transitions: 348.3/62.4, 326.3/62.4, 300.3/62.4, 328.3/62.4, and 379.3/287.3 for anandamide, OEA, PEA, SEA, and 2-AG, respectively. For the corresponding internal standards, we used the following transitions: 352.3/62.4,

330.3/62.4, 304.3/62.4, 331.3/62.4, and 384.3/287.3 for anandamide-d4, OEA-d4, PEA-d4, SEA-d3, and 2-AG-d5, respectively. The linearity of the measuring ranges was assessed with standard curves ranging from 1 to 25 nM for anandamide and 10 to 500 nM for OEA, PEA, and SEA, and 50 to 1250 nM for 2-AG in duplicate. The linearity of the standard curves was $R^2 \geq 0.9$ for all analytes. Isotopic dilution was used for quantification of the analytes, performed according to their area ratio of their corresponding deuterated internal standard signal area. Linear regression and χ^2 weighting were applied. Undetected levels were considered as 0 nM. Xcalibur® (version 2.1, Thermo Scientific) software was used for peak integration and quantification.

Statistics. Data analyses were perfomed using the IBM SPSS version 22.0 (IBM Corporation, Route 100 Somers, NY) and the GraphPad Prism computer programmer version 6.03 (GraphPad Software Inc. San Diego, CA). Two-way ANOVA with repeated measures on time (two timepoints) were used for comparisons of data between and within group's pre and post the exercise program, and for comparison of lipid levels in MD (five time-points) between and within groups. Usual physical activity levels were analyzed using Mann-Whitney tests.

Multivariate data analysis (MVDA) using the software SIMCA 15.0 (Umetrics, Umeå, Sweden) was applied. Principal component analysis (PCA) was used for data overview and for multivariate correlation analysis and the SIMCA tools Hotelling's T^2 and distance to model in X-space were used to identify strong and moderate multivariate outliers, respectively.

For all MVDA, data were log-transformed when needed using the auto transform function, and scaling to unit variance was applied (27). The parameters R^2 and Q^2 diagnostic were used to evaluate model quality. R^2 describes the goodness of fit—the fraction of sum of squares of all the variables explained by a principal component. Q^2 describes the goodness of prediction—the fraction of the total variation of the variables that can be predicted by a principal component using CV methods. A $P \leq 0.05$ was used as level of significance in all statistical analyses.

RESULTS

Valid for the primary cohort: the exercise participation in the 15-wk intervention was 83% for FM and 89% for HC. The usual physical activity levels was assessed before the exercise program (measured with Leisure Time Physical Activity Instrument) and HC estimated a statistically significant higher level of moderate and vigorous usual physical activity per week ($P < 0.05$) than FM. Mean ± SD age in years was 50.1 ± 10.5 for FM and 50.3 ± 12.8 for HC and mean body mass index (BMI) ± SD was 26.5 ± 4.7 $kg \cdot m^{-2}$ for FM and 24.4 ± 4.4 $kg \cdot m^{-2}$ for HC. No statistically significant difference in age or BMI was found between groups. Valid for the secondary cohort: mean age was 53.3 ± 11.7 yr, and mean BMI was 28.1 ± 4.6 $kg \cdot m^{-2}$ for FM and 55.3 ± 5.3 yr and 24.6 ± 4.1 $kg \cdot m^{-2}$ for HC, respectively.

The results concerning clinical parameters are valid for the primary cohort which are described in detail in the Participants section.

Clinical Parameters

These data have essentially been presented elsewhere, however not on the exact same number of participants (21,22). Mean values with SD in brackets for VAS, PPT, FIQ, HAD-A, HAD-D, MFI, and physical capacity scorings; hand grip force, isometric elbow-flexion force, and isometric knee-extension force before and after the intervention, are shown in Table 1. At baseline, VAS, FIQ, HAD-A, HAD-D, and MFI were significantly higher in FM compared to HC. The PPT and the physical capacity measures hand grip force and isometric elbow flexion were significantly lower in FM compared with HC at baseline. In FM VAS, FIQ, HAD-D, and MFI were significantly decreased, and hand grip force and isometric elbow force significantly increased after the intervention. In HC, solely hand grip force was increased significantly after the intervention. After the intervention all parameters remained different between groups in the same way as before the intervention, except for the isometric elbow force scorings, which did not differ significantly between FM and HC after the intervention (Table 1).

Plasma Levels of Lipid Mediators in FM and HC Pre and Post Resistance Exercise

No significant differences in plasma lipid levels existed between FM and HC at baseline except for SEA levels, which were elevated in FM compared with CON ($P = 0.04$) (Table 2). After 15 wk of resistance exercise, anandamide levels increased significantly ($P = 0.01$) in FM (Table 2). Anadamide levels were significantly higher ($P = 0.03$) and SEA were lower ($P = 0.05$) in FM compared with controls after the exercise program (Table 2).

Bivariate Correlation between Lipid Plasma Levels Preintervention and Postintervention

Pre the intervention. Significant positive correlations ($r > 0.5$, $P < 0.01$) between all the NAE except for AEA and SEA existed in FM. In HC, significant correlations existed between OEA, PEA, and SEA ($r > 0.5$, $P < 0.01$), and AEA correlated significantly with PEA ($r = 0.36$, $P < 0.05$). Correlations coefficients for the lipids in FM and HC before the physical exercise are shown in Table 3.

Post the intervention. Similar as pre intervention, significant positive correlations existed between the NAE ($r > 0.37$, $P < 0.05$) (except for between AEA and SEA) in both FM and HC. Correlations coefficients for the lipids in FM and HC before the physical exercise are shown in Table 3. Moreover, significant positive correlations between AEA and 2-AG ($r > 0.4$, $P < 0.05$) existed after the intervention in both FM and HC which was the most prominent change in lipid level interrelationship illustrated with scatterplots in Figure 1.

TABLE 1. Clinical parameters.

Main Effects of ANOVA				
Measure	**df**	**Within Subjects**	**Between Subjects**	**Interaction (Time–Group)**
VAS (0–100)	(1, 68)	n.s.	$P \leq 0.001$, $F = 105.2$, p. $\eta 2 = 0.607$	$P = 0.003$, $F = 9.80$, p. $\eta 2 = 0.126$
PPT (kPa)	(1, 68)	n.s.	$P \leq 0.001$, $F = 70.6$, p. $\eta 2 = 0.509$	n.s
FIQ (0–100)	(1, 68)	n.s.	$P \leq 0.001$, $F = 266.5$, p. $\eta 2 = 0.814$	$P = 0.014$, $F = 6.41$, p. $\eta 2 = 0.095$
HAD-A (0–21)	(1, 68)	n.s.	$P \leq 0.001$, $F = 33.0$, p. $\eta 2 = 0.340$	n.s
HAD-D (0–21)	(1, 68)	n.s.	$P \leq 0.001$, $F = 47.6$, p. $\eta 2 = 0.426$	$P = 0.013$, $F = 6.58$, p. $\eta 2 = 0.093$
MFI (20–100)	(1, 68)	$P = 0.027$, $F = 5.08$, p. $\eta 2 = 0.069$	$P \leq 0.001$, $F = 188.4$, p. $\eta 2 = 0.735$	$P = 0.037$, $F = 4.56$, p. $\eta 2 = 0.063$
HG force (N)	(1, 68)	$P \leq 0.001$, $F = 17.59$, p. $\eta 2 = 0.205$	$P \leq 0.001$, $F = 17.56$, p. $\eta 2 = 0.205$	n.s
IEF force (N)	(1, 60)	n.s	$P \leq 0.014$, $F = 6.37$, p. $\eta 2 = 0.096$	$P = 0.004$, $F = 8.90$, p. $\eta 2 = 0.129$
IKE force (N)	(1, 68)	$P = 0.012$, $F = 6.68$, p. $\eta 2 = 0.089$	n.s	n.s

Pairwise Comparisons within Groups						
Measure	**FM Pre**	**FM Post**	***P***	**HC Pre**	**HC Post**	***P***
VAS (0–100)	48.7 (22.9)	37.1 (24.2)	0.001*	1.9 (5.9)	4.5 (14.2)	0.422
PPT (kPa)	170 (71)	178 (80)	0.731	359 (96)	365 (183)	0.804
FIQ (0–100)	62.1 (13.8)	54.9 (19.2)	0.004*	7.3 (8.0)	8.9 (12.2)	0.527
HAD-A (0–21)	8.8 (4.7)	7.9 (5.2)	0.171	1.5 (1.8)	1.9 (2.4)	0.832
HAD-D (0–21)	7.7 (4.2)	6.5 (4.6)	0.007*	3.1 (3.0)	3.0 (2.9)	0.409
MFI (20–100)	78.9 (11.9)	68.2 (12.6)	0.002*	35.7 (12.4)	35.5 (13.6)	0.934
HG force (N)	167 (70)	178 (71)	0.008*	223 (40)	236 (43)	0.002*
IEF force (N)	12.9 (4.9)	14.7 (5.3)	0.001*	17.3 (3.7)	16.8 (4.1)	0.426
IKE force (N)	343 (88)	361 (99)	0.089	342 (106)	365 (104)	0.059

Pairwise Comparisons between Groups						
Measure	**FM Pre**	**HC Pre**	***P***	**FM Post**	**HC Post**	***P***
VAS (0–100)	48.7 (22.9)	1.9 (5.9)	0.001*	37.1 (24.2)	4.5 (14.2)	0.001*
PPT (kPa)	170 (71)	359 (96)	0.001*	178 (80)	365 (183)	0.001*
FIQ (0–100)	62.1 (13.8)	7.3 (8.0)	0.001*	54.9 (19.2)	8.9 (12.2)	0.001*
HAD-A (0–21)	8.8 (4.7)	1.5 (1.8)	0.001*	7.9 (5.2)	1.9 (2.4)	0.001*
HAD-D (0–21)	7.7 (4.2)	3.1 (3.0)	0.001*	6.5 (4.6)	3.0 (2.9)	0.001*
MFI (20–100)	78.9 (11.9)	35.7 (12.4)	0.001*	68.2 (12.6)	35.5 (13.6)	0.001*
HG force (N)	167 (70)	223 (40)	0.001*	178 (71)	236 (43)	0.001*
IEF force (N)	12.9 (4.9)	17.3 (3.7)	0.001*	14.7 (5.3)	16.8 (4.1)	0.184
IKE force (N)	343 (88)	342 (106)	0.996	361 (99)	365 (104)	0.885

Main effects of ANOVA and pairwise comparisons within and between 37 FM and 33 HC. Mean values with SD in brackets for of pain intensity (VAS), hand grip (HG) force, isometric elbow-flexion (IEF) force, and isometric knee-extension (IKE) force pre and a post 15-wk resistance exercise intervention. *P* values were adjusted for multiple comparisons with Bonferroni (note: the maximal isometric elbow-flexion force measures were conducted in 25/37 FM subjects). Mean values with (SD) in brackets and statistical *P* values are displayed. These data have essentially been presented elsewhere, however, not the exact same number of participants.
*Statistically significant.

Relationship between Plasma 2-AG and AEA—An Explorative Ratio Analysis

Because not only the linear relationship between 2-AG and AEA appear to increase after the exercise program in both FM and HC (Fig. 1) but also because there are conflicting result regarding levels of AEA and 2-AG—both in respect not only to their relation to physical exercise but also to their relation to FM—an explorative analysis of the 2-AG/AEA ratio was performed as an attempt to capture the merged value of this two main eCB, a value which has the potential to reflect the excess of CB receptor signaling more broadly than the separate levels.

Interestingly a statistically significant decrease in 2-AG/AEA ratio existed in FM (ratios expressed with means and SD) (pre = 66.6 [61.1]; post = 37.8 [27.5]) postexercise, $P = 0.002$ compared with a nonsignificant increase in HC (pre = 47.3 [27.0]; post = 57.7 [37.3]), $P = 0.192$. Furthermore, a statistically significant difference existed between FM and HC postexercise $P = 0.014$, (Fig. 2).

Relationships between Plasma Lipid Mediators and Selected Clinical Parameters Pre and Post 15-Wk Resistance Exercise Program in FM

To investigate whether different correlation patterns existed between the five lipids in plasma and the selected clinical parameters in FM and HC pre and post the exercise program PCA was used.

First multivariate outliers were checked using 11 variables (the five lipids, VAS, HAD-D, FIQ, MFI, elbow-flexion force, hand grip force) for all subjects. As intended one pre ($R^2 = 0.71$ and $Q^2 = 0.37$) and one post model ($R^2 = 0.72$ and $Q^2 = 0.34$) and in both were identified two strong outliers and eight moderate outliers. The two strong outliers belonged to FM and were removed. No abnormal data were identified in the moderate outliers, and hence, they were retained in the subsequent analyses.

In the following step, PCA for the preexercise and postexercise, respectively, including the above mentioned 11 variables of the FM group were performed (pre: $R^2 = 0.50$ and $Q^2 = 0.004$; post: $R^2 = 0.50$ and $Q^2 = 0.006$). Loading scatter plots (i.e., the relationships between variables) of these two analyses are illustrated in Figure 3.

Figure 3 (pre exercise) illustrates a pattern with three groups of variables. Hence, the NAE (but not 2-AG)—as also reported above according to the bivariate correlations—intercorrelate in the third quadrant, and VAS, HAD-D, FIQ, and MFI scores intercorrelate in the fourth quadrant. Moreover, isometric elbow-flexion force, hand grip force, and 2-AG formed a third group of variables. It is evident from Figure 3 that isometric elbow-flexion force and hand grip force

TABLE 2. Main effects of two-way ANOVA and pairwise comparisons within and between groups of lipid concentrations in plasma before and after 15-wk resistance exercise intervention.

Main Effects of ANOVA								
Lipid	***df***	**Within Sub.**			**Between Sub.**		**Interaction (Time–Group)**	
AEA	(1, 68)	n.s			n.s		$P = 0.006$, $F = 7.94$, partial η2 = 0.105	
OEA	(1, 68)	n.s			n.s		n.s	
PEA	(1, 68)	n.s			n.s		n.s	
SEA	(1, 68)	n.s			n.s		$P = 0.003$, $F = 9.42$, partial η2 = 0.125	
2-AG	(1, 68)	n.s			n.s		n.s	
Pairwise Comparisons within Groups								
Lipid	**FM Pre**	**FM Post**	***P***	**Power**	**HC Pre**	**HC Post**	***P***	**Power**
AEA	0.26 (0.13)	0.34 (0.18)	**0.01***	0.96	0.31 (0.16)	0.25 (0.14)	**0.14**	0.89
OEA	5.66 (1.92)	5.33 (2.11)	**0.38**	0.25	5.39 (1.29)	6.06 (1.96)	**0.09**	0.78
PEA	8.51 (2.03)	7.96 (2.69)	**0.23**	0.41	8.72 (1.63)	9.18 (2.54)	**0.35**	0.26
SEA	2.56 (1.18)	2.05 (1.10)	**0.03***	0.96	2.05 (0.83)	2.57 (1.06)	**0.03***	0.98
2-AG	12.8 (8.49)	11.5 (8.03)	**0.36**	0.25	12.5 (3.97)	12.6 (6.17)	**0.92**	0.05
Pairwise Comparisons between Groups								
Lipid	**FM Pre**	**HC Pre**	***P***	**Power**	**FM Post**	**HC Post**	***P***	**Power**
AEA	0.26 (0.13)	0.31 (0.16)	0.12	0.50	0.34 (0.18)	0.25 (0.14)	**0.03***	0.90
OEA	5.66 (1.92)	5.39 (1.29)	**0.50**	0.16	5.33 (2.11)	6.06 (1.96)	**0.14**	0.53
PEA	8.51 (2.03)	8.72 (1.63)	**0.63**	0.10	7.96 (2.69)	9.18 (2.54)	**0.06**	0.76
SEA	2.56 (1.18)	2.05 (0.83)	**0.04***	0.79	2.05 (1.10)	2.57 (1.06)	**0.05***	0.78
2-AG	12.8 (8.49)	12.5 (3.97)	**0.83**	0.24	11.5 (8.03)	12.6 (6.17)	**0.51**	0.14

Main effects of ANOVA and pairwise comparisons within and between groups of plasma levels of AEA, OEA, PEA, SEA) and 2-AG in nM (SD) collected from 37 women with FM and 33 HC pre and post a 15-wk resistance exercise intervention. *P* values were adjusted for multiple comparisons with Bonferroni. Power were calculated with $\alpha = 0.05$. *P* values in bold emphasis report the statistical difference between samples collected pre compared with post the intervention in FM and HC, and the difference between FM and HC pre and post the intervention.
*Statistically significant.

correlated negatively with pain intensity, HAD-D, FIQ, and MFI according to the second component of the PCA.

Postexercise (Fig. 3), the principal patterns between the variables remained. However, isometric elbow-flexion force and hand grip force were stronger correlated with 2-AG postexercise (i.e. less distance indicate stronger correlation). Moreover, after the intervention 2-AG are inversely correlated with pain intensity, HAD-D, FIQ, and MFI.

Levels of NAE in Microdialysate from the Vastus Lateralis Muscle

Result concerning pain aspects during the MD experiments has essentially been reported elsewhere (28) and was not the scope in this study.

PEA, OEA, and SEA were analyzed in MD samples from vastus lateralis muscle at baseline (140 min after MD catheter insertion), immediately after muscle work (160 min) and for the following 1-h recovery (180–220 min). The level ranges for OEA, PEA, and SEA, respectively were: 0.0 to 8.7 nM; 0.0 to 1.3 nM; 0.0 to 3.4 nM in FM and 0.0 to 4.6 nM; 0.0 to 2.5 nM; 0.0 to 10.4 nM in HC at the five time points (140–220). Plots of concentrations of OEA, PEA, and SEA from time point 140 to 220 min are attached as supplemental digital content (see Figure, Supplemental Digital Content 1, levels of NAE in dialysate from vastus lateralis muscle, http://links.lww.com/MSS/B928). Neither significant within-group nor group differences existed for the lipid concentrations.

DISCUSSION

The principal findings in this study are as follows:

- Plasma anandamide levels were increased in FM after a 15-wk resistance exercise program.
- After the program, plasma SEA was decreased in FM but increased in HC.
- In a multivariate context, muscle strength aspects were associated with plasma 2-AG in FM after the exercise program.

The outcomes of the 15-wk resistance exercise intervention at group level in FM were statistically reduced pain intensity, depression, FM impact, fatigue, and increased muscle strength (these data have essentially been presented elsewhere, however, not on the exact same number of participants [21,22]). In the present study, we analyzed the effect on this intervention on plasma level changes of EC and N-acylethanolamines, and to the best of our knowledge, this is the first report concerning these lipid mediators and chronic effects of resistance exercise in FM.

However, concerning the acute effect on plasma eCB and NAE in healthy humans, there are several reports regarding

TABLE 3. Bivariate correlations of plasma concentrations of lipid mediators before and after 15-wk resistance exercise.

FM	**AEA**	**OEA**	**PEA**	**SEA**	**2-AG**	**AEA**	**OEA**	**PEA**	**SEA**	**2-AG**
AEA	1	0.57**	0.56**	0.32	0.07	1	0.45**	0.43**	0.15	0.41*
OEA		1	0.71**	0.53**	–0.14		1	0.76**	0.70**	0.19
PEA	A		1	0.55**	0.12	B		1	0.76**	0.23
SEA				1	0.17				1	0.17
2-AG					1					1
HC	**AEA**	**OEA**	**PEA**	**SEA**	**2-AG**	**AEA**	**OEA**	**PEA**	**SEA**	**2-AG**
AEA	1	0.18	0.36*	0.25	–0.17	1	0.55**	0.56**	0.26	0.41*
OEA		1	0.68**	0.66**	0.17		1	0.74**	0.37*	0.24
PEA	C		1	0.50**	0.15	D		1	0.57**	0.32
SEA				1	0.16				1	0.08
2-AG					1					1

Interrelation between the lipids in plasma expressed with *r* values. In FM, before (panel A) and after (panel B), and in HC before (panel C) and after (panel D) a 15-wk resistance exercise.
*Significance level 0.05, and **0.01.

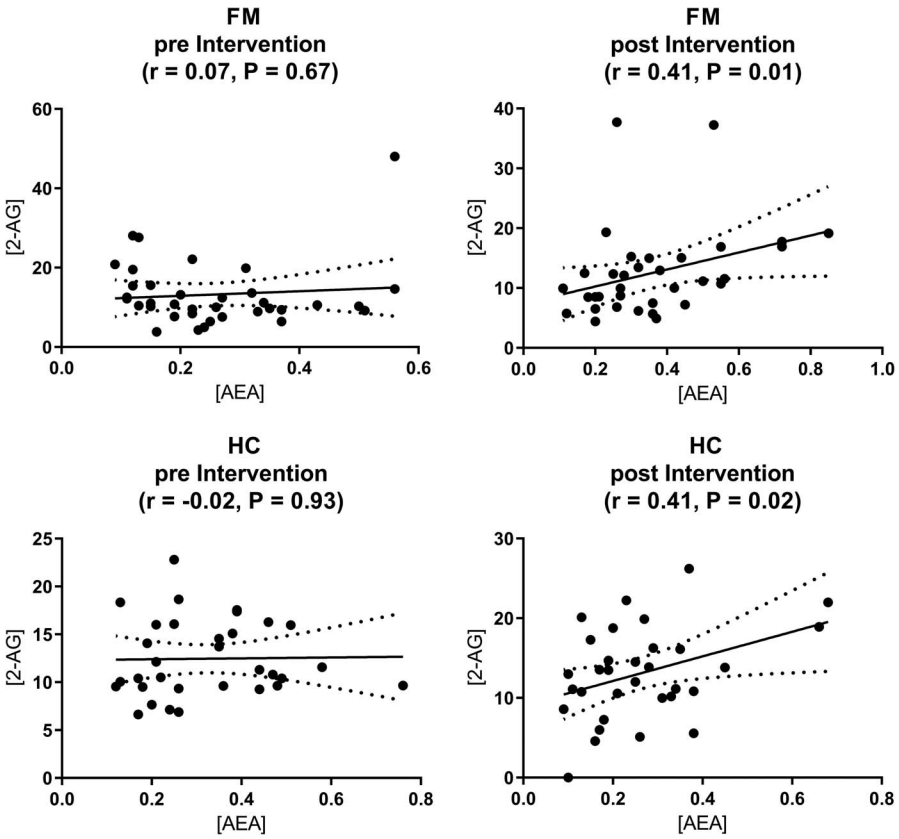

FIGURE 1—The linear relationships between plasma AEA and 2-AG levels were increased both in FM and HC after the intervention which is illustrated with scatterplots and best fitted linear regression with 95% confidence interval (*dotted*) pre and post the intervention for FM in the upper part and for HC in the lower part of the figure.

aerobic exercise. Raichlen et al (16) reported significantly increased anandamide levels in response to 30 min of running, but not walking, in humans (n = 10) (and dogs [n = 8]). Anandamide and BDNF increased significantly and were correlated in healthy trained male cyclists (n = 11) after 60 min of cycle exercise, and anandamide were suggested to be important for the neuroplastic and antidepressant effects of exercise (17). There are fewer report concerning resistance exercise; however, Koltyn et al (19) investigated the effect of 3 min submaximal isometric exercise in healthy humans (29 women and 29 men) which induced hypoalgesia and resulted in significantly increased plasma levels of AEA, OEA, PEA, DHEA, 2-AG, and 2-OG.

Regarding chronic effects of physical exercise on these lipid mediators, there are fewer reports.

Di Marzo et al (29) reported decreased plasma anandamide and 2-AG levels in viscerally obese men (n = 49) following a 1-yr lifestyle modification program. Gasperi et al (30) did not report any difference in plasma anandamide, 2-AG and PEA in physically active men (n = 8) compared with age-matched sedentary subjects (n = 8).

In the present investigation, basal plasma anandamide did not differ between FM and HC, which is in line with our previous report (14), but not with the report from Kaufman et al (13). After the exercise intervention plasma anandamide increased significantly in FM and was significantly higher compared with HC. No clear association between anandamide and pain, depression, fatigue, general health status, or muscle strength was found neither before nor after the exercise program in this study (Fig. 3).

Concerning the origin of blood levels of anandamide, it has been reported that it originates from different organs and

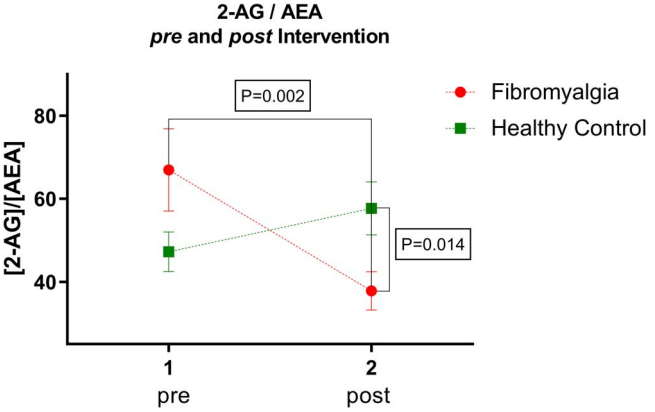

FIGURE 2—Ratio levels with error bars in SEM of plasma 2-AG and AEA in FM and HC participant's pre and post 15 wk physical exercise program. Statistically significant differences between FM and HC and between pre and post are shown with *P* values.

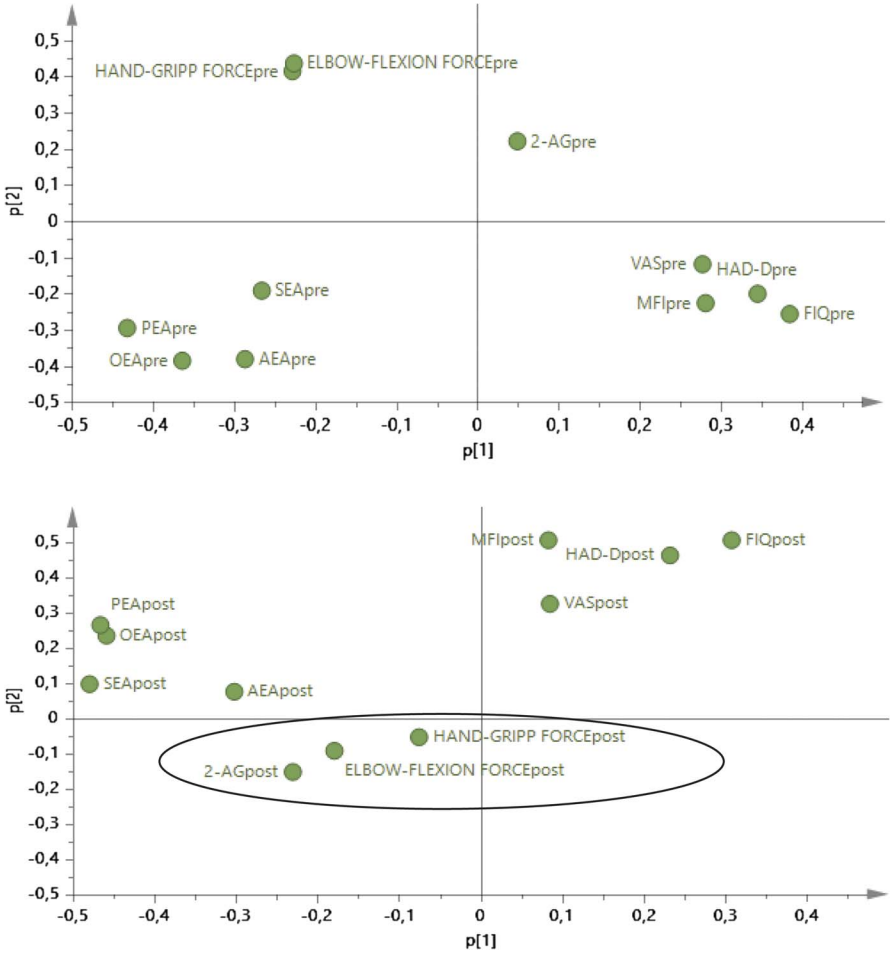

FIGURE 3—PCA loading plot displaying the multivariate correlations *preexercise* (the upper plot) and *postexercise* (the lower plot) between the plasma lipids and certain clinical variables. Variables with high absolute loadings are important for a certain component. Variables on opposite sides of the plot origin, in diagonally opposed quadrants are negatively correlated. Variables located near each other are positively correlated. The main difference between the two plots is highlighted within the ellipse in the *postexercise* loading plot.

tissues, including brain, muscle, adipose tissue, and circulating cells as an indirect marker of tissue EC tone (31), the interpretation of the change of plasma AEA on mechanistic levels are therefore challenging. However, global pain intensities and depression scorings significantly decreased in FM but not pain sensitivity, which could indicate that central rather than peripheral pain mechanisms, and depression are involved, where cannabinoid receptors and AEA could play important roles (the role of the EC in depression, reward, and pain was reviewed by Huang et al (32)). A more clinical interpretation could be that chronic resistance exercise reduces depression and pain in FM, and the increased plasma anandamide has the potential—by partly originating from the brain—to reflect neurogenesis and plastic changes in the brain and activation of descending pain pathways.

Concerning the activation of pain pathways, in a recent animal study on inflammatory pain, Dos Santos et al (33) suggest that anandamide activation of spinal CB_2 receptors and reduction of activated microglia are involved in exercise-induced antinociception.

For plasma SEA, there was almost an opposite relationship (compared with anandamide) that was, before the intervention SEA was higher in FM compared with HC, and after the intervention, it was lower in FM compared with HC. The lower usual activity level in FM compared with HC before the intervention might contribute to the different in baseline plasma SEA levels. Furthermore, after the exercise intervention, plasma SEA was significantly increased in HC, but significantly decreased in FM. This is interestingly a change in the same directions as in the previous report from Ghafouri et al (20) were locally sampled trapezius muscle SEA levels decreased after acute 20 min low-intensity muscle work in women with chronic widespread pain. In that study, participants repeated 30-cm movements of an 11.8-g peg back and forth on a pegboard at a speed of 1.3 Hz for 20 min, which significantly increased local pain intensities measures in CWP ($P = 0.001$). In the present study, in comparison, women with FM performed a 20-min physical task by repetitively extending the knee 15° to 20° by slowly lifting the leg from a resting position to a straight position (0°). Each repetition lasted for 5 s, without any rest between repetitions. This task did also significantly increase local pain intensity in FM ($P < 0.001$) which was reported in (28). No significant muscle SEA levels change existed due to this muscle work; however, a tendency of decreased muscle SEA levels could be observed after time

point 160 min, see the supplementary figure (Fig. S1, http://links.lww.com/MSS/B928). Possible explanations for the discrepancies in these two studies are a slight difference in diagnosis (CWP vs FM), different muscles (trapezius vs vastus lateralis), and different intensities of the performed muscle work.

Furthermore, SEA has like AEA been found both centrally and peripherally in animals (34); however, the knowledge about its endogenous role is more limited. Both Dalle Carbonaro et al and Berdyshev et al (11) have reported of its acute anti-inflammatory characteristics in animal models, but it has also been suggested by Terrazzino et al. to have anorexic effects, and to reduce food intake in mice. We have previously reported basal levels of plasma SEA which were significantly higher in women with FM (n = 104) compared with HC (n = 116) (14). However, we have also reported about unaltered basal plasma SEA concentrations from women with CWP (n = 17) compared with controls (n = 21), a result in line with a report from Hellström et al (35) who also investigated basal plasma SEA in women with CWP (n = 15) and controls (n = 27). This indicates that high basal plasma SEA are more associated with FM than with CWP, which suggests that widespread pain are not the main reason for elevated plasma SEA levels but instead other manifestations of FM, and it cannot be ruled out that it reflects ongoing low-grade inflammatory processes, which has been proposed to proceed in FM. No clear association between SEA levels and pain, depression, fatigue, general health status, or muscle strength existed before or after the exercise program in this study (Fig. 3).

The significant decrease in FM and increase in HC of plasma SEA after 15-wk resistance exercise in this study might reflect different metabolic processes derived from different tissues, for example, muscles and brain; however, this need to be further investigated in future studies. To the best of our knowledge, this is the first report about effects of chronic resistance exercise on plasma SEA levels in not only FM subjects but also in HC.

These results might be signs of improved—or altered—metabolic health," and plasma AEA and SEA might be potential biomarkers to reflect beneficial metabolic effects of physical exercise both centrally and peripherally. The result could potentially be helpful in the clinic when evaluating therapeutic exercise programs and informing patients of the beneficial effects of physical exercise. However, our results need to be confirmed in future studies of patients with FM, and the levels of the investigated substances need to be explored also in other chronic pain conditions after exercise interventions.

There is biological importance in the increased linear relation between AEA and 2-AG in both FM and HC (Fig. 1), but at the same time clearly diverging ratio (2-AG/AEA) between the two groups (Fig. 2). Possibly, the increased association between AEA and 2-AG could reflect regular chronic effects of physical exercise. The diverging ratio of 2-AG/AEA could instead point to EC metabolic irregularities between FM and HC, which discloses after chronic physical exercise. The ratio analysis was exploratory with the hypothesis that the merged value has the potential to reflect the excess of CB receptor signaling more broadly.

From previous research, it is known that AEA and 2-AG are promiscuous ligands and activate several molecular targets besides the CB receptors. Both AEA and 2-AG have affinity for the transient receptor potential vanilloid-1 receptor, and the PPAR receptors and 2-AG have been recognized to directly activate gamma-aminobutyric acid A ($GABA_A$) receptor (36). They can also be biosynthesized and inactivated independent of each other. In addition to the roles of modulating processes of pain, inflammation, and stress, the EC are playing a pivotal role for energy homeostasis in CNS and in peripheral tissues and organs (37), and not only CB receptors but also transient receptor potential vanilloid-1 receptors are expressed in human skeletal muscle, and 2-AG has been suggested to play a crucial role in the control of myotube formation (38). Moreover, exercise-induced muscular contraction stimulates A-delta and C nociceptive fibers and has been shown to result in the activation of endogenous analgesia mechanisms (39). However, people with FM generally have decreased muscle strength which suggests impaired muscle function (40), and nociceptive input from the muscle is proposed to be one peripheral source of pain (2).

In the present study (in a MVDA context), plasma 2-AG levels were associated with muscle strength (in hands and arms) in FM to a higher extent after the exercise program (Fig. 3). Moreover, even though no significant change in 2-AG levels existed after the intervention, negative associations between 2-AG and pain intensity, HAD-D, FIQ, and MFI (Fig. 3) were apparent after the intervention. Unfortunately, no valid MVDA models for HC solely were obtainable, which precluded a comparison. However, in the models where both FM and HC were included, no clear association between 2-AG and muscle strength was found pre or post the exercise program, which indicate that this increased relationship was more prominent for FM. One interpretation of this result is that plasma 2-AG are reflecting muscle anabolism in FM to a higher extent than in HC. In Stensson et al (14), we reported about an inverse association between 2-AG and pain sensitivity in FM, this relation could not be confirmed in this investigation.

Circulating eCB and NAE levels has been reported to be influenced by age, BMI, diet and by food consumption. In this study, no significant correlation between the five lipids and age or BMI was found in FM, and no difference in age or BMI existed between groups in the primary cohort. Diet was not controlled for in this study which is a limitation. Blood and microdialysis sampling were not fully standardized, sampling occurred both AM and PM without fasting prescriptions, which is another limitation. El-Talatini et al (41) reported that AEA levels vary during the menstrual cycle and peaking in the ovulation phase. This was not controlled for in this study, neither were premenopause versus postmenopause effects on lipid levels controlled, which further limits the interpretation of the results.

In conclusion, after a 15-wk resistance exercise program (exercise performed twice a week), plasma anandamide increased and plasma SEA decreased on group level in women with FM. In HC, on the contrary, plasma SEA increased after the program. This might point to chronic effects of resistance exercise on levels

of these lipid mediators and could potentially reflect beneficial metabolic effects of resistance exercise both centrally and peripherally; however, this needs to be confirmed in future studies.

In FM, pain and depression scores decreased after the exercise program, but there were no associations between these changes and the altered lipid levels.

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