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Analysis of gene expression pattern reveals potential targets of dietary oleoylethanolamide in reducing body fat gain in C3H mice☆

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Abstract

Oleoylethanolamide (OEA) has been previously reported to regulate food intake and body weight gain when administered intraperitoneally. Nevertheless, little information is available with regard to oral administration. To assess whether oral OEA can also exert a similar effect on body fat, we fed C3H mice a high-fat diet supplemented with either 10 or 100 mg/kg body weight OEA for 4 weeks. OEA supplementation significantly lowered food intake over the 4 weeks and decreased adipose tissue mass. Plasma triglyceride levels were also significantly decreased by OEA treatment. In order to identify the potential molecular targets of OEA action, we screened the expression levels of 44 genes related to body fat mass and food intake in peripheral tissues. Adipose tissue fatty acid amide hydrolase (FAAH), intestinal fatty acid transporter/cluster of differentiation 36 and the OEA receptor G-protein-coupled receptor 119 (GPR119) were among the most OEA-responsive genes. They were also associated with reduced body fat pads regardless of the dose. Adipose FAAH was found to be primarily associated with a decrease in food intake. Our data suggest that the anti-obesity activity of OEA partially relies on modulation of the FAAH pathway in adipose tissue. Another mechanism might involve modulation of the newly discovered GPR119 OEA signaling pathway in the proximal intestine. In conclusion, our study indicates that oral administration of OEA can effectively decrease obesity in the mouse model and that modulation of the endocannabinoid fatty acid ethanolamide pathway seems to play an important role both in adipose tissue and in small intestine.

Keywords: Oleoylethanolamide; PLS regression; Diet-induced obesity; Endocannabinoids

1. Introduction

Fatty acid ethanolamides (FAEAs) or *N*-acyl-ethanolamides are structurally related lipids that contain a fatty acid moiety linked to ethanolamine [1]. FAEAs are a family of natural lipids found in plant and animal tissues. They have several effects on health, including regulation of energy balance and control of food intake, and they also possess anti-inflammatory properties [2]. FAEAs are also formed *in vivo* from N-acetylated phosphatidyl-ethanolamide derivatives. Some FAEAs such as anandamide (*N*-arachidonoyl-ethanolamine) and *N*-oleoylethanolamide (OEA) are found in the brain, in biological tissues and in neuronal cells [1]. OEA is also found in low amounts in foodstuffs and is mainly produced by endogenous synthesis [1].

In rodents, intraperitoneal administration of OEA was reported to induce satiety and peripheral utilization of lipid substrates, thereby leading to reduction in body fat gain [2]. *In vitro* studies and knockout animal models have suggested some mechanisms of action, such as the involvement of peroxisome proliferator-activated receptor (PPAR) α signaling [3], fatty acid transporter/cluster of differentiation 36 (FAT/CD36)-dependent lipid uptake by the proximal intestine [4], selected neuronal activation [5] and ghrelin signaling [6]. The proximal intestine seems to be a target organ for satiety control [2]. Indeed, it has been shown that OEA regulates food intake in wild-type mice, but not in PPARα(−/−) mice. OEA levels in the proximal intestine are regulated by nutritional state and are increased in obese rats [7], underlying the link between OEA biosynthesis, food intake control and

Abbreviations: ACO, acyl-CoA oxidase; FAAH, fatty acid amide hydrolase; FAT/CD36, fatty acid transporter/cluster of differentiation 36; FIAF, fasting-induced adipocyte factor; GPR119, G-protein-coupled receptor 119; PPAR, peroxisome proliferator-activated receptor.

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obesity. It was recently shown that OEA can also bind to the G-protein-coupled receptor 119 (GPR119) [8]. When administered intraperitoneally, OEA reduces food intake by influencing several feeding pattern parameters, decreasing meal size, delaying intake of the first meal and increasing intervals between meals [9]. The effects of oral OEA administration have also been examined 24 h after acute force-feeding administration, and it has been shown to significantly decrease food intake over the first 12 h [9]. Nevertheless, these studies were performed over short periods of time (6 h to 11 days for intraperitoneal administration and 24 h for oral administration), and long-term effects on satiety remain unknown.

In this study, the effects of long-term (4 weeks) oral administration of OEA on body weight gain and cumulative food intake were investigated in mice. Supplementation was considered to be chronic in this study because it was three times longer than subchronic studies performed over 7 days [4] or 11 days [3]. In these studies, the effects of OEA on food intake and body weight gain were significant. A multigene screening approach was carried out to investigate the effects of chronic oral OEA administration on representative peripheral pathways that are potentially implicated in food intake and lipid metabolism. This study could help delineate potential targets for further studies.

2. Materials and methods

2.1. Animals, diet and experimental design

All experiments were conducted in accordance with the French Regulations for Animal Experimentation (Article 19, October 1987, Ministry of Agriculture) after approval by our institutions' referee for animal care. Adult male C3H mice at 8 weeks of age were provided by Janvier Elevage (Le Genest-St-Isle, France). C3H mice were chosen because this strain was shown to develop as much adiposity as [10] or more adiposity than [11] C57Bl6J mice when exposed to a high-fat diet. After arrival, the mice were individually housed and maintained for 2 weeks on different diets and water *ad libitum*. Twenty-one mice were divided into three groups (n=7 per group). The experimental groups were sorted prior to treatment based on average weight. Mice were then fed a high-fat diet (lipids represented 50% of daily energy) for 2 weeks. The composition of the high-fat diet per kilogram was as follows: 284.5 g of corn starch, 89.5 g of saccharose, 250 g of casein, 50 g of cellulose, 10 g of a mix of vitamins (V1001; HPPS, California, USA) [100 g of vitamin premix contains 40,000 IU of vitamin A, 10,000 IU of vitamin D3, 500 IU of vitamin E, 5 mg of menadione sodium bisulfite (62.5% menadione), 2 mg of biotin (1%), 100 μg of cyanocobalamin (0.1%), 20 mg of folic acid, 300 mg of nicotinic acid, 160 mg of calcium pantothenate, 70 mg of pyridoxine HCl, 60 mg of riboflavin and 60 mg of thiamin HCl], 35 g of a mix of minerals (S10026; HPPS) (100 g of mineral premix contains 14.9 g of Ca, 11.4 g of P, 1.4 g of Mg, 10.3 g of K, 0.9 g of S, 2.9 g of Na, 4.6 g of Cl, 5.7 mg of Cr, 17.1 mg of Cu, 0.6 mg of I, 128.6 mg of Fe, 168.6 mg of Mn, 0.5 mg of Se and 82.9 mg of Zn) and 281 g of canola oil (UPAE, Jouy en Josas, France). OEA was then added to the diets at different levels to provide 0, 10 and 100 mg/kg body weight OEA. This corresponded to 0, 36.3 and 363 mg OEA/kg food for each diet at the beginning of the experiment. During the chronic supplementation period, OEA supplementation was recalculated weekly as a function of the average body weights of mice in each group. The mice were fed this OEA diet for 4 weeks. During the nutritional intervention, daily food intake was monitored, and the mice were weighed three times a week.

2.2. Sampling

At the end of the experimental period, mice were sacrificed by drawing of blood after they had been anesthesized with isoflurane (Abbot France, Rungis, France). Plasma was obtained by centrifugation (1000×g for 10 min at 4°C). Mesenteric, epididymal, inguinal and peritoneal adipose depots, as well as liver, stomach, small intestine mucosa and gastrocnemius muscles, were excised and frozen in liquid nitrogen. Plasma and organ samples were kept at −80°C until analysis. Triglycerides, glucose, total cholesterol and high-density lipoprotein cholesterol were directly measured in plasma samples collected during the sacrifice by enzymatic procedures using a Beckman Coulter Systems SYNCHRON LX 20 (Beckman Coulter, Fullerton, CA, USA) (oxidase method, Beckman Coulter for glucose; GPO method, Beckman Coulter for triglyceride; oxidase and esterase method, Beckman Coulter for cholesterol). The values are expressed in millimoles per liter.

2.3. RNA extraction and gene expression study

Total RNAs from liver, small intestine epithelium (first 10 cm), adipose tissue, gastrocnemius muscle and stomach were extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instruction. RNA concentration was determined spectrophotometrically. For DNA synthesis, reverse transcription was performed with Superscript II (Invitrogen) in accordance with the manufacturer's protocol. Real-time quantitative PCR was performed on cDNA, as described previously, using Stratagene Mx 3005P (Stratagene, Cedar Creek, TX, USA) and SYBR Green Master mix kit (Eurogentec, Philadelphia, PA, USA). Values were expressed as ratios of RNA levels relative to one control mouse (diets with 0 mg/kg body weight OEA) using $\Delta\Delta(C_t)$ [12] in duplicate.

2.4. Statistical analysis

Results are presented as mean±standard error of the mean (S.E.M.). Statistical analysis of physiological parameters and gene expression data was performed by one-way analysis of variance (ANOVA) on Statview software (SAS Institute, Cary, NC, USA). Statistical significance was set at $P \leq .05$. Multivariate statistics were performed using SIMCA P-11 (Umetrix, Umea, Sweden) after unit of variance or Pareto scaling of the variables. The choice of scaling unit was determined by calculation of the best prediction values following cross-validation (Q^2 values). Characterization of the effects of OEA on multigenic response was examined with an orthogonal projection on latent structure discriminant analysis (PLS-DA) using the OEA dose in the diet as class determinant (Y dummy variables). In addition, we examined the relationship between gene expressions (X variables) and adipose tissue fat pad masses or cumulative food intake (Y variables) using a similar PLS regression. These methods are based on principal components. We used the orthogonal-signal-corrected PLS-DA procedure and the orthogonal-signal-corrected PLS procedure (Fig. 1) that allow variations of the X variables that are not correlated to Y to be filtered out. This makes the orthogonally treated data more precise and easier to interpret [13]. The relevance of individual PLS regression coefficients (center-scaled coefficients) to the multigenic response was determined by jackknifing (a resampling technique) [14] using 99% confidence intervals. The contribution of each gene to the multigenic response was also checked by calculating the variable importance in projection (VIP) coefficients using a cutoff of 1 and a 99% jackknife confidence interval. This multivariate approach has had previous applications and validations in similar nutritional studies [15–18]. As a general rule, each model is internally validated by cross-validation. In this procedure, part of the data is kept out of model development and then predicted by the model and compared with actual values. This was repeated until all subjects had been left out once. The number of latent variables (principal components) yielding the lowest percentage of misclassifications (error rate) was chosen as the optimal model. For each model, seven rounds of left-out subject combinations were performed. More precisely, response values (class membership for PLS-DA; cumulated food intake and adipose fat pad mass for PLS) for the excluded mice were predicted by the model and compared with the actual values. In this test, the predicted values (Q^2, fraction of the total variation of the response Y that can be predicted) should be close to the actual values (R^2), with predicted values (Q^2) of >0.5 (50%). Our PLS-DA components could model 74% of the gene expression variations (R^2X) and 98.7% of the dietary group multigenic response (R^2Y) (prediction value Q^2=82.5% after cross-validation). The PLS regression components could model 99.6% (97% in the first component) of the total variation in cumulative food intake (R^2Y), with a cumulative prediction of 99% after cross-

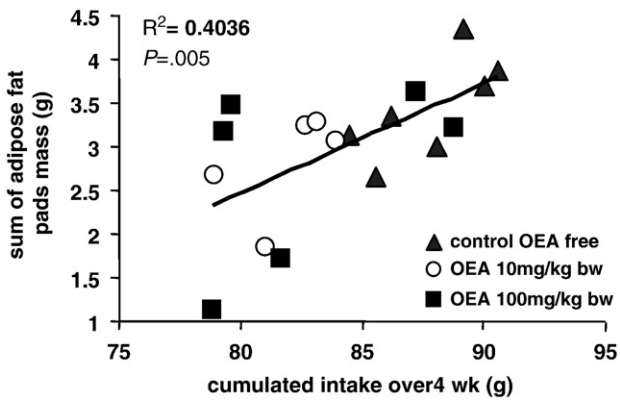

Fig. 1. Relationships among cumulative food intake over 4 weeks and the sum of adipose fat pads (epididymal, inguinal, retroperitoneal and mesenteric). n=5–7 mice per group.

Table 1
Physiological parameters of mice fed the experimental diets for 4 weeks

Physiological parameters	OEA dose (mg/kg body weight)		
	0	10	100
Cumulative food intake (g)	82.6±0.2[a]	77.2±0.3[b]	77.1±0.3[b]
Liver weight (g)	1,4±0.02[a]	1.2±0.01[b]	1.4±0.04[a]
Total adipose tissue (g)	3.43±0.22[a]	2.63±0.29[b]	2.72±0.42[b]
Mesenteric adipose tissue (g)	0.78±0.07	0.73±0.06	0.72±0.08
Epididymal adipose tissue (g)	1.22±0.07[a]	0.89±0.1[b]	1±0.16
Inguinal adipose tissue (g)	0.95±0.1[a]	0.66±0.1[b]	0.7±0.13[b]
Peritoneal adipose tissue (g)	0.48±0.03[a]	0.35±0.05[b]	0.3±0.03[b]
Body weight (g)	33.5±0.9	31.5±0.4	31.17±1
Gastrocnemius muscle	0.09±0.01[a]	0.14±0.01[b]	0.13±0.01[b]
Plasma triglycerides (mmol/L)	1.59±0.35[a]	0.44±0.14[b]	0.66±0.14[b]
Plasma total cholesterol (mmol/L)	2.94±0.21[a]	2.5±0.16	2.43±0.21[b]

Results are presented as mean±S.E.M. (n=6–7 mice per group). Values in the same row and not sharing the same superscript letter (a and b) are statistically different (P<.05). Total adipose tissue refers to the sum of mesenteric, epididymal, inguinal and peritoneal adipose fat pads.

validation (Q^2 values). The respective values for predicting adipose fat mass variations were R^2Y=92.7% (all variations explained in the first component) and Q^2=90%.

In addition, a correlation network was built using the open-source freeware Cytoscape (http://cytoscape.org/) [19] in order to determine the strongest pairwise interactions among the genes participating in the multigenic response [15].

3. Results

3.1. Food intake

According to linear regression analysis, cumulative food intake over the experimental period could predict up to 40% of the total variation in adipose fat pad masses (Fig. 1). Interestingly, food intake was slightly (−6.5%) but significantly (P<.05) decreased in mice consuming OEA (Table 1). Daily food intakes were significantly different for both doses of OEA when compared to controls over the entire experimental period (two-way ANOVA, P<.01); however, the observed effect was not dose dependent.

3.2. Physiological and biochemical parameters

OEA treatment did not induce hepatomegaly (Table 1). OEA induced a similar decrease in total adipose fat pad weight. Peritoneal adipose tissue was mainly decreased, and inguinal fat pads, taken as an indicator of subcutaneous fat depot (main site of fat storage), were also decreased by OEA intake (P≤.05), whereas mesenteric fat pads remained unaffected by the dose administered. The final body weights of the mice were not affected either (Table 1). A significant increase in gastrocnemius muscle weight was observed in both OEA groups when compared to controls (Table 1).

Plasma triglycerides were significantly lower in both OEA groups (−72%, P≤.05 for the 10-mg dose; −59%, P≤.05 for the 100-mg dose) (Table 1). A trend to decreased plasma total cholesterol was observed at 10 mg/kg, which reached statistical significance upon treatment at 100 mg/kg body weight.

3.3. Gene expression analysis

The effects of oral OEA administration on gene expression were evaluated via an advanced pattern recognition statistical procedure (orthogonal-signal-corrected PLS-DA) (Fig. 2). This allows the overall gene expression of each mouse to be linearly reduced to one score and the contribution of each gene in this score to be determined. The effect of treatments on the gene expression pattern can thus be easily measured, and the most responsive genes can be identified. We also determined, by the same procedure, how the multigenic response to OEA feeding was predictive of some phenotypic outcomes such as food intake and adipose fat pad mass and, from this, which individual genes among all the genes analyzed contributed most to these outcomes.

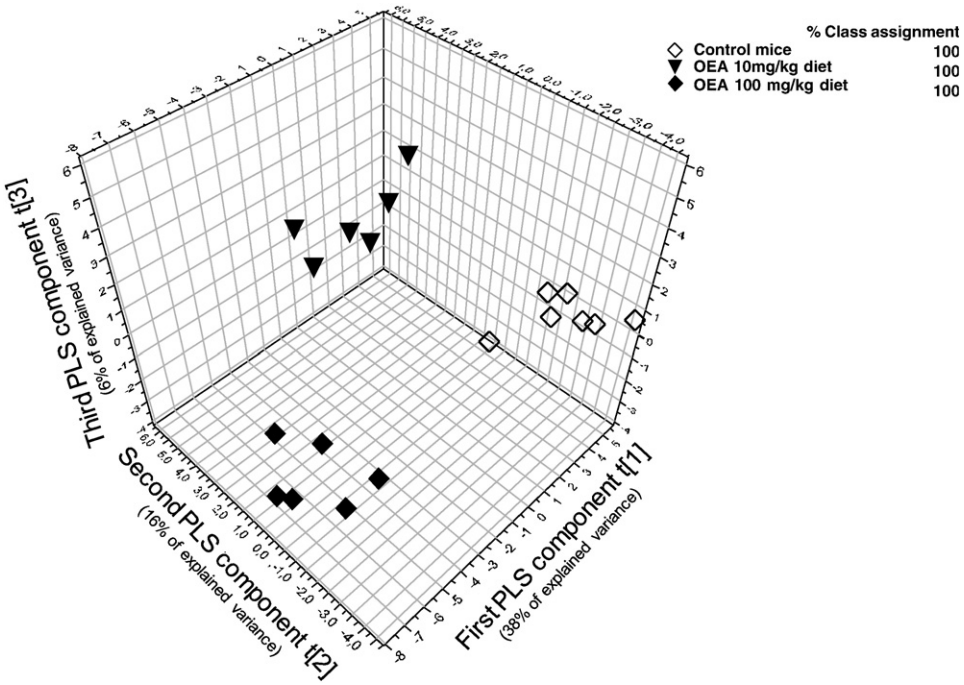

Fig. 2. Discriminant analysis plot (orthogonal-signal-corrected PLS-DA) showing the diet-induced multigenic response (44 genes per mouse) of mice given 10 mg/kg body weight OEA, 100 mg/kg body weight OEA or OEA-free high-fat diet (control). The specificity of the multigenic response allowed a 100% class assignment of mice into their respective treatment group. The genes that contributed most to the individual score values and group assignments are displayed in Fig. 4. n=6–7 mice per group.

First, the multigenic response to both OEA treatments can be visualized as a three-dimensional plot showing a dose-related response (Fig. 2). Our model explained up to 76% of the total variance in gene expression. From this analysis, a common set of OEA-responsive genes can be extracted from the multigenic response, regardless of the dose (e.g., 10 mg or 100 mg/kg body weight) (Fig. 3). These were the intestinal lipid transporter FAT/CD36, the OEA satiety receptor GPR119 (up-regulation) and the endocannabinoid hydrolase fatty acid amide hydrolase (FAAH) in adipose tissue (up-regulation). Nevertheless, the multigenic response in OEA-supplemented mice can also be dose differentiated from that in OEA-free mice. The 100-mg/kg dose can be best characterized by a decreased expression in the muscle acyl-CoA oxidase (*ACO*) gene and then by an increase in the expression of other liver and muscle lipid oxidative genes of the intestinal FAAH (endocannabinoid metabolism) and fasting-induced adipocyte factor (FIAF) (inhibition of adipose tissue fatty acid transport). Conversely, the 10-mg/kg OEA dose was best characterized by an increased expression of both adipose tissues visfatin and adiponectin (insulin signaling) and FIAF (inhibition of adipose tissue fatty acid transport).

These multigenic response values, reduced to one score value for each mice, were then plotted to the corresponding food intake values and adipose fat pad masses and found to be highly predictive of both phenotypic outcomes (R^2=.97 and .93, respectively) (Fig. 3A and B). From this, we calculated which genes contributed most to phenotypic outcome predictions (Fig. 4). In this situation, adipose tissue *FAAH*, the gene most influenced by both OEA treatments (Fig. 2), was also by far the most influential gene in determining the relationships among multigenic response, food intake and adipose masses (Fig. 4). Conversely, the OEA intestinal satiety receptor *GPR119*, another OEA-responsive gene up-regulated at both doses, could only be predictive of the decrease in adipose fat pad masses. Adipose tissue visfatin (up-regulated at 10 mg/kg) and muscle ACO (down-regulated at 100 mg/kg) also contributed to this relationship. Adipose tissue visfatin (up-regulation) also significantly contributed to the multigenic response to food intake.

In addition to PLS analysis, we performed a pairwise correlation analysis of the gene expression data. With the Cytoscape tool, a correlation network was plotted in which the most important gene–gene interactions could be highlighted for various treatments. Such analysis gives further insight into the interactions of OEA-responsive genes. Under the stringent conditions applied (Pearson correlation coefficient over or equal to .7), 29 of the 44 analyzed genes demonstrated strong pairwise relationships in which the larger interactions network included 16 genes (Fig. 5). The adipose tissue FAAH was connected to a larger number of immediate neighbors such as GPR119, CD36, FIAF and cannabinoid receptor 1 (intestine), ACO and uncoupling protein 2 (muscle) and acyl-CoA carboxylase (liver).

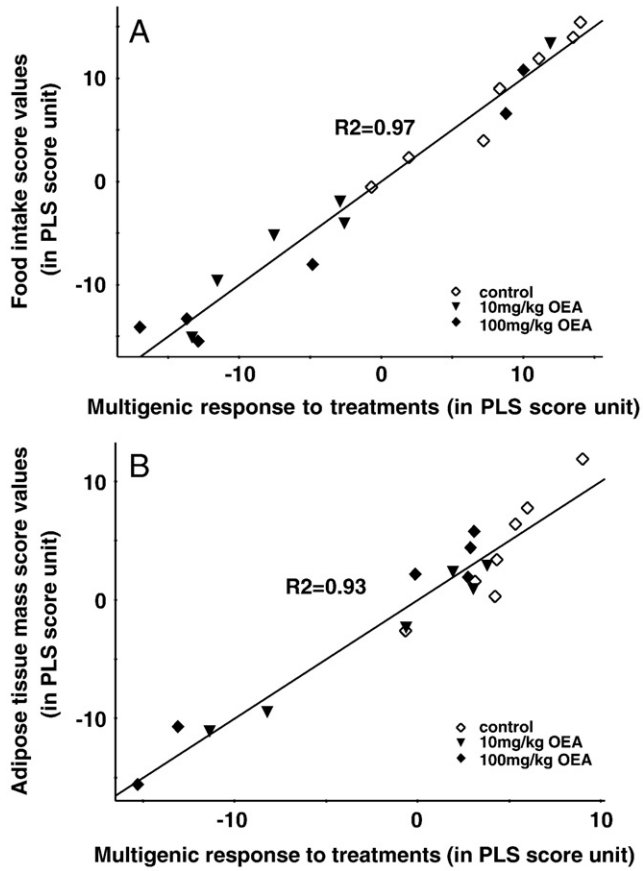

Fig. 3. (A) Relationships among the multigenic responses reduced to one value per mouse by PLS analysis and the corresponding cumulative food intake as calculated by PLS regression. (B) Relationships among the multigenic responses reduced to one value per mouse by PLS analysis and the corresponding adipose fat pad masses as calculated by PLS regression. Individual gene contributions to prediction are displayed in Fig. 4. Groups are indicated by symbols. n=6–7 mice per group.

4. Discussion

This is the first study to examine the effect of chronic OEA feeding on body fat mass. Our main purpose was thus mainly exploratory. Our purpose was, firstly, to establish a possible fat-lowering effect of oral OEA and, secondly, to find potential molecular targets at the gene expression level. For this, we used a multigene screening approach examining together and separately 44 genes potentially involved in food intake and adipose tissue mass control. This helped to determine what would be the main possible molecular targets accompanying OEA effects. The multigenic response was examined by a multivariate statistical approach, which also allowed ranking of the contribution of each gene to the response [17].

As previously observed with intraperitoneal OEA administration [3,20], body fat mass and plasma triglyceride levels were reduced upon oral OEA supplementation. This indicates that OEA remains biologically active when given orally for a long period of time.

Compared to controls, chronic oral OEA exposure at both doses significantly modified the multigenic response pattern, which can also be associated with both food intake and adipose tissue fat pad masses. In this pattern, the adipose tissue *FAAH* gene appeared to be an important target of oral OEA, since it showed the most responses to treatment among all the genes and it also appeared most inversely related to both food intake and adipose tissue masses. Furthermore, this gene appeared pivotal since it correlated with most of the genes that were OEA sensitive and associated with both adipose fat mass variation and food intake.

Among these OEA-responsive genes, the genes encoding the intestinal transporter FAT/CD36, the satietogenic OEA receptor GPR119 and the FAAH in adipose tissue were the set of genes that were most commonly regulated regardless of the OEA dose. In a previous study [3], chronic intraperitoneal administration of OEA induced the intestinal expression of PPARα and FAT/CD36. We observed that FAT/CD36 was up-regulated in the same manner in our oral administration study. We also found a statistically significant up-regulation of intestinal PPARα, as previously described, after intraperitoneal infusion [3,21], but at the lowest dose only (10 mg/kg). It is also possible that this transcription factor is

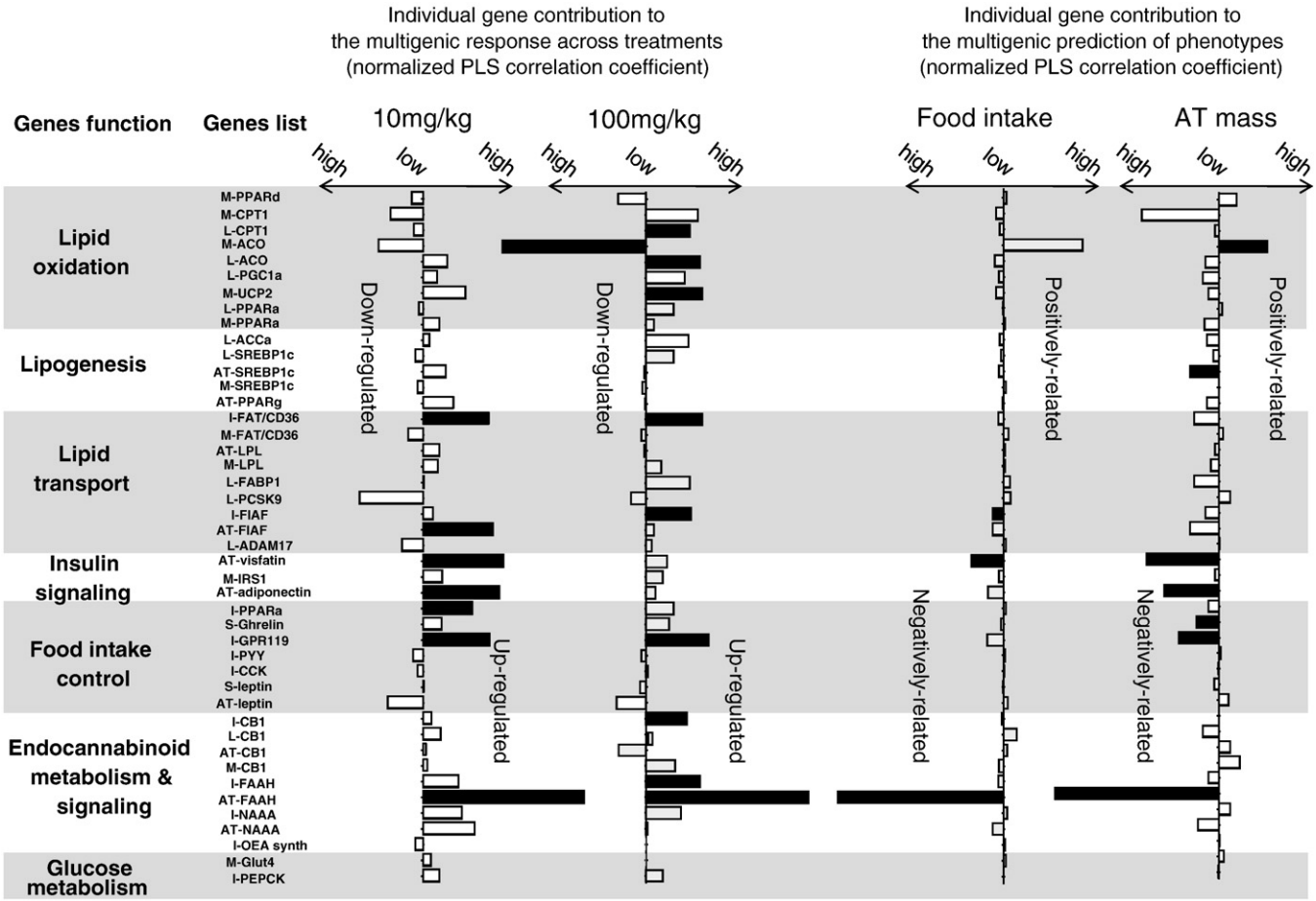

Fig. 4. Individual gene contribution to the multigenic response of mice given 10 or 100 mg/kg body weight OEA, as displayed in Fig. 2, or to the multigenic response predicting either cumulative food intake or body fat pad masses, as displayed in Fig. 3. Black histograms indicate genes showing a significant contribution to either class assignment (mice treated with 10 mg or 100 mg/kg body weight) or phenotypic outcomes (cumulative food intake or adipose fat pad mass), as determined by jackknifing with 99% confidence intervals. Values are normalized to the adipose tissue FAAH PLS score. $n=6$–7 mice per group. Genes are displayed by physiological functions. I: proximal intestine; M: muscle; L: liver; AT: adipose tissue; S: stomach.

activated posttranscriptionally by oral OEA. This is suggested by the up-regulation of *FAT/CD36*, a specific PPARα target gene in the proximal intestine [22], and also observed in an *in vitro* assay (reviewed in O'Sullivan [23]).

Interestingly, supplementation with 100 mg/kg body weight OEA did not reduce either fat storage or food intake more than the 10 mg/kg body weight supplementation. Nevertheless, it induced a larger up-regulation of the FAEA degradation pathways (*FAAH* gene expression) in the intestine. This would represent a negative feedback mechanism by OEA on its own bioavailability and thereby partly explain why we did not observe a larger decrease in adipose fat pad masses at 100 mg/kg body weight than at 10-mg/kg body weight. In addition, it can be hypothesized that there is an indirect effect of the up-regulation of this degradation pathway on the intestinal degradation of other fatty acyl-ethanolamides such as anandamide [24]. The implication of the fatty acyl-ethanolamide endocannabinoid degradation pathway in obesity has been recently investigated [25]. The expression of the gene encoding FAAH was more down-regulated in the adipose tissue of obese patients than in the adipose tissue of lean individuals. This down-regulation was accompanied by higher fatty acyl-ethanolamide concentrations in obese subjects [26]. In this study, we found that up-regulation of the *FAAH* gene in adipose tissue was also strongly negatively associated with adipose fat pad masses. Therefore, it is possible that OEA induces the peripheral fatty acyl-ethanolamide degradation pathway, especially in adipose tissue, thus modulating the levels of these endocannabinoids in this tissue [27]. This hypothesis can be further investigated using FAAH knockout mice along with FAEA concentration assessment in plasma.

OEA has recently been shown to be a ligand [28] and intestinal activator [29] of a new receptor, GPR119. Long-term activation of this receptor by the selective agonist PSN632408 reduced cumulative food intake and body fat deposition in rats [8]. This is the first time that this receptor has also been shown to be up-regulated by OEA feeding. We also found that its expression level was inversely correlated to adipose fat pad masses.

In conclusion, we have shown for the first time that 4 weeks of a diet enriched with OEA decreased both cumulative food intake and adipose fat mass deposition in mice fed a high-fat diet. This study confirmed that OEA remains biologically active when administered orally over a long period of time. Our multigene screening approach identified potential molecular targets of OEA. Among these targets is the gene encoding the adipose tissue endocannabinoid degradation enzyme FAAH, which seems to play a central role in our experimental conditions. Others included the OEA-activated receptor GPR119, the lipid transporter FAT/CD36 and the *FAAH* genes, all located in the small intestine and strongly associated with the adipose tissue FAAH. This study suggests that the biological action of OEA could be mediated through modulation of the FAEA

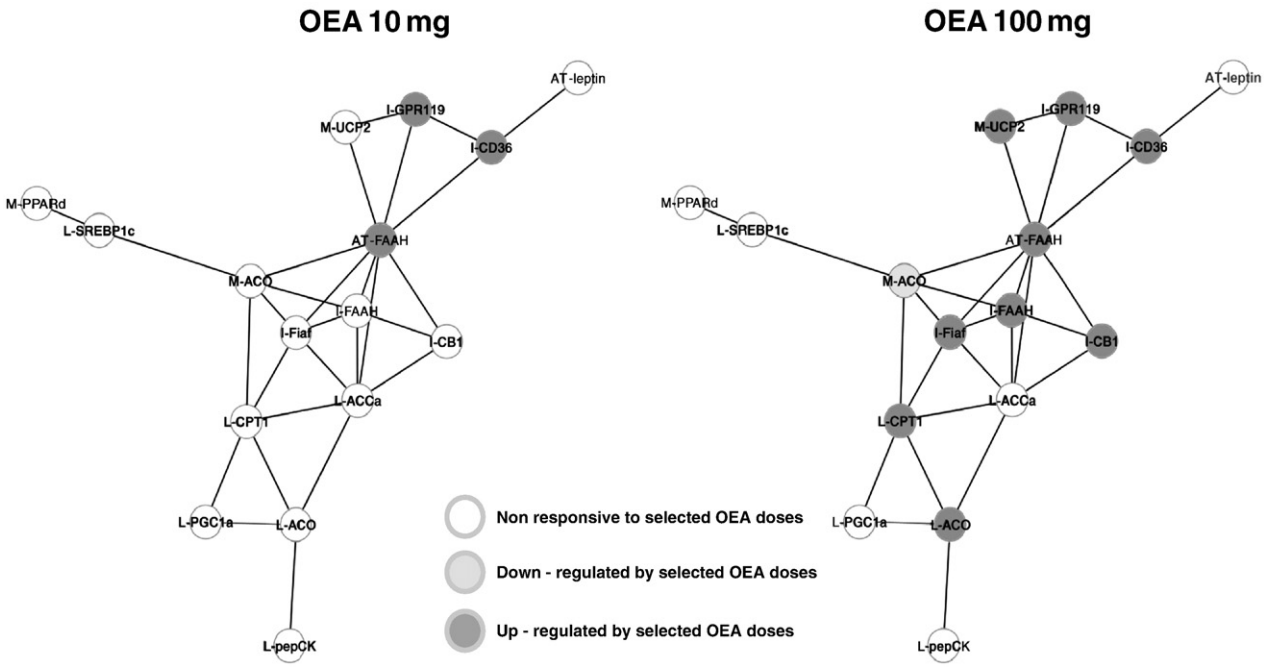

Fig. 5. Correlation plots indicating pairwise correlations between genes analyzed by quantitative PCR that have a Pearson correlation coefficient over or equal to .7 (R^2=.5). Only the strongest relationships remained with this stringent filtering (16 over 44 genes can be visualized). White and progressive gray colorings indicate nonresponsive, negatively responsive or positively OEA-responsive genes, respectively, in any dietary intervention group, and they can be linked to Fig. 4. n=6–7 mice per group. I: proximal intestine; M: muscle; L: liver; AT: adipose tissue; S: stomach.

degradation pathway, especially in adipose tissue. Another mechanism of action might rely on the modulation of the newly discovered GPR119 OEA signaling pathway. The direct impact of OEA on these targets needs some mechanistic confirmation. A gene invalidation or interference approach might help to give us further insights into these questions.

Acknowledgments

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References

[1] Di Marzo V, Sepe N, De Petrocellis L, Berger A, Crozier G, Fride E, et al. Trick or treat from food endocannabinoids? Nature 1998;396:636–7.
[2] Thabuis C, Tissot-Favre D, Bezelgues JB, Martin JC, Cruz-Hernandez C, Dionisi F, et al. Biological functions and metabolism of oleoylethanolamide. Lipids 2008;43:887–94.
[3] Fu J, Gaetani S, Oveisi F, Lo Verme J, Serrano A, Rodriguez De Fonseca F, et al. Oleoylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-alpha. Nature 2003;425:90–3.
[4] Yang Y, Chen M, Georgeson KE, Harmon CM. Mechanism of oleoylethanolamide on fatty acid uptake in small intestine after food intake and body weight reduction. Am J Physiol Regul Integr Comp Physiol 2007;292:R235–41.
[5] Ahern GP. Activation of TRPV1 by the satiety factor oleoylethanolamide. J Biol Chem 2003;278:30429–34.
[6] Cani PD, Montoya ML, Neyrinck AM, Delzenne NM, Lambert DM. Potential modulation of plasma ghrelin and glucagon-like peptide-1 by anorexigenic cannabinoid compounds, SR141716A (rimonabant) and oleoylethanolamide. Br J Nutr 2004;92:757–61.
[7] Izzo AA, Piscitelli F, Capasso R, Marini P, Cristino L, Petrosino S, et al. Basal and fasting/refeeding-regulated tissue levels of endogenous PPAR-alpha ligands in Zucker rats. Obesity (Silver Spring) 2009.
[8] Overton HA, Babbs AJ, Doel SM, Fyfe MC, Gardner LS, Griffin G, et al. Deorphanization of a G protein-coupled receptor for oleoylethanolamide and its use in the discovery of small-molecule hypophagic agents. Cell Metab 2006;3:167–75.
[9] Oveisi F, Gaetani S, Eng KT, Piomelli D. Oleoylethanolamide inhibits food intake in free-feeding rats after oral administration. Pharmacol Res 2004;49:461–6.
[10] West DB, Boozer CN, Moody DL, Atkinson RL. Dietary obesity in nine inbred mouse strains. Am J Physiol 1992;262:R1025–32.
[11] Alexander J, Chang GQ, Dourmashkin JT, Leibowitz SF. Distinct phenotypes of obesity-prone AKR/J, DBA2J and C57BL/6J mice compared to control strains. Int J Obes (London) 2006;30:50–9.
[12] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method. Methods 2001;25:402–8.
[13] Trygg J, Vold S. Orthogonal projection to latent structure. J Chemom 2002;16:119–28.
[14] Chavance M. Jackknife and bootstrap. Rev Epidemiol Sante Publique 1992;40:209–18.
[15] de Roos B, Rucklidge G, Reid M, Ross K, Duncan G, Navarro MA, et al. Divergent mechanisms of *cis*9,*trans*11- and *trans*10,*cis*12-conjugated linoleic acid affecting insulin resistance and inflammation in apolipoprotein E knockout mice: a proteomics approach. FASEB J 2005;19:1746–8.
[16] Ala-Korpela M. Critical evaluation of ^{1}H NMR metabonomics of serum as a methodology for disease risk assessment and diagnostics. Clin Chem Lab Med 2008;46:27–42.
[17] Navarro V, Portillo MP, Margotat A, Landrier JF, Macarulla MT, Lairon D, et al. A multi-gene analysis strategy identifies metabolic pathways targeted by *trans*-10,*cis*-12-conjugated linoleic acid in the liver of hamsters. Br J Nutr 2009:1–9.
[18] Martin J-C, Canlet C, Delplanque B, Agnani G, Lairon D, Gottardi G, et al. ^{1}H-NMR plasma metabonomics can differentiate the early atherogenic potential of dairy products in the hyperlipidemic hamster. Atherosclerosis 2009:127–33.
[19] Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003;13:2498–504.
[20] Fu J, Oveisi F, Gaetani S, Lin E, Piomelli D. Oleoylethanolamide, an endogenous PPAR-alpha agonist, lowers body weight and hyperlipidemia in obese rats. Neuropharmacology 2005;48:1147–53.
[21] Guzman M, Lo Verme J, Fu J, Oveisi F, Blazquez C, Piomelli D. Oleoylethanolamide stimulates lipolysis by activating the nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR-alpha). J Biol Chem 2004;279:27849–54.
[22] Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev 1999;20:649–88.
[23] O'Sullivan SE. Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. Br J Pharmacol 2007;152:576–82.
[24] Petersen G, Sorensen C, Schmid PC, Artmann A, Tang-Christensen M, Hansen SH, et al. Intestinal levels of anandamide and oleoylethanolamide in food-deprived

rats are regulated through their precursors. Biochim Biophys Acta 2006;1761: 143–50 discussion 1–2.
[25] Engeli S, Bohnke J, Feldpausch M, Gorzelniak K, Janke J, Batkai S, et al. Activation of the peripheral endocannabinoid system in human obesity. Diabetes 2005;54:2838–43.
[26] Di Marzo V. The endocannabinoid system in obesity and type 2 diabetes. Diabetologia 2008;51:1356–67.
[27] Matias I, Di Marzo V. Endocannabinoids and the control of energy balance. Trends Endocrinol Metab 2007;18:27–37.
[28] Brown AJ. Novel cannabinoid receptors. Br J Pharmacol 2007;152:567–75.
[29] Lauffer LM, Iakoubov R, Brubaker PL. GPR119 is essential for oleoylethanolamide-induced glucagon-like peptide-1 secretion from the intestinal enteroendocrine L-cell. Diabetes 2009:640–6.