



**HAL**  
open science

## Metabolic and psychiatric effects of acyl coenzyme A binding protein (ACBP)/diazepam binding inhibitor (DBI)

Adrien Joseph, Stéphanie Moriceau, Valentina Sica, Gerasimos Anagnostopoulos, Jonathan Pol, Isabelle Martins, Antoine Lafarge, Maria Chiara Maiuri, Marion Leboyer, Joséphine Loftus, et al.

### ► To cite this version:

Adrien Joseph, Stéphanie Moriceau, Valentina Sica, Gerasimos Anagnostopoulos, Jonathan Pol, et al.. Metabolic and psychiatric effects of acyl coenzyme A binding protein (ACBP)/diazepam binding inhibitor (DBI). *Cell Death and Disease*, 2020, 11 (7), pp.502. 10.1038/s41419-020-2716-5 . hal-02927426

**HAL Id: hal-02927426**

**<https://hal.science/hal-02927426>**

Submitted on 26 May 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

ARTICLE

Open Access

# Metabolic and psychiatric effects of acyl coenzyme A binding protein (ACBP)/diazepam binding inhibitor (DBI)

Adrien Joseph<sup>1,2,3</sup>, Stéphanie Moriceau<sup>4</sup>, Valentina Sica<sup>1,2,5</sup>, Gerasimos Anagnostopoulos<sup>1,2,3</sup>, Jonathan Pol<sup>1,2</sup>, Isabelle Martins<sup>1,2,6</sup>, Antoine Lafarge<sup>1,2,3</sup>, Maria Chiara Maiuri<sup>1,2</sup>, Marion Leboyer<sup>7,8,9,10</sup>, Josephine Loftus<sup>7,11</sup>, Frank Bellivier<sup>7,12</sup>, Raoul Belzeaux<sup>7,13,14</sup>, Fabrice Berna<sup>7,15</sup>, Bruno Etain<sup>7,12</sup>, Delphine Capdevielle<sup>7,16</sup>, Philippe Courtet<sup>7,17,18</sup>, Caroline Dubertret<sup>7,19</sup>, Julien Dubreucq<sup>7,20</sup>, D' Amato Thierry<sup>7,21</sup>, Guillaume Fond<sup>7,22,23</sup>, Sebastien Gard<sup>7,24</sup>, Pierre-Michel Llorca<sup>7,25</sup>, Jasmina Mallet<sup>7,19</sup>, David Misdrahi<sup>7,24</sup>, Emilie Olié<sup>7,17</sup>, Christine Passerieux<sup>7,26</sup>, Mircea Polosan<sup>7,27</sup>, Paul Roux<sup>7,26</sup>, Ludovic Samalin<sup>7,25</sup>, Franck Schürhoff<sup>7,8,9,10</sup>, Raymond Schwan<sup>1,28</sup>, the FACE-SZ and FACE-BD (FondaMental Academic Centers of Expertise, for Schizophrenia and for Bipolar Disorder) Groups, Christophe Magnan<sup>29</sup>, Franck Oury<sup>4</sup>, José M. Bravo-San Pedro<sup>30</sup> and Guido Kroemer<sup>1,2,31,32,33</sup>

## Abstract

Acyl coenzyme A binding protein (ACBP), also known as diazepam binding inhibitor (DBI) is a multifunctional protein with an intracellular action (as ACBP), as well as with an extracellular role (as DBI). The plasma levels of soluble ACBP/DBI are elevated in human obesity and reduced in anorexia nervosa. Accumulating evidence indicates that genetic or antibody-mediated neutralization of ACBP/DBI has anorexigenic effects, thus inhibiting food intake and inducing lipocatabolic reactions in mice. A number of anorexigents have been withdrawn from clinical development because of their side effects including an increase in depression and suicide. For this reason, we investigated the psychiatric impact of ACBP/DBI in mouse models and patient cohorts. Intravenously (i.v.) injected ACBP/DBI protein conserved its orexigenic function when the protein was mutated to abolish acyl coenzyme A binding, but lost its appetite-stimulatory effect in mice bearing a mutation in the  $\gamma 2$  subunit of the  $\gamma$ -aminobutyric acid (GABA) A receptor (GABA<sub>A</sub>R). ACBP/DBI neutralization by intraperitoneal (i.p.) injection of a specific mAb blunted excessive food intake in starved and leptin-deficient mice, but not in ghrelin-treated animals. Neither i.v. nor i.p. injected anti-ACBP/DBI antibody affected the behavior of mice in the dark–light box and open-field test. In contrast, ACBP/DBI increased immobility in the forced swim test, while anti-ACBP/DBI antibody counteracted this sign of depression. In patients diagnosed with therapy-resistant bipolar disorder or schizophrenia, ACBP/DBI similarly correlated with body mass index (BMI), not with the psychiatric diagnosis. Patients with high levels of ACBP/DBI were at risk of dyslipidemia and this effect was independent from BMI, as indicated by multivariate analysis. In summary, it appears that ACBP/DBI neutralization has no negative impact on mood and that human depression is not associated with alterations in ACBP/DBI concentrations.

Correspondence: José M. Bravo-San Pedro ([josemabr@ucm.es](mailto:josemabr@ucm.es)) or Guido Kroemer ([kroemer@orange.fr](mailto:kroemer@orange.fr))

<sup>1</sup>Centre de Recherche des Cordeliers, Equipe labellisée par la Ligue Contre le Cancer, Université de Paris, Sorbonne Université, Inserm U1138, Paris, France

<sup>2</sup>Metabolomics and Cell Biology Platforms, Institut Gustave Roussy, Villejuif, France

Full list of author information is available at the end of the article

These authors contributed equally: Adrien Joseph, Stéphanie Moriceau

The members of consortium group are listed below the Acknowledgements section

Edited by G.M. Fimia

© The Author(s) 2020



**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

## Introduction

Acyl coenzyme A (CoA) binding protein (ACBP) has been identified as an ubiquitously expressed 86 amino acid polypeptide that binds medium-sized (C<sub>14</sub>–C<sub>22</sub>) acyl CoA chains in the cytoplasm of multiple (if not all) cell types<sup>1</sup>. In addition, this protein acts as an “endozepine”

and displaces benzodiazepines such as tritium-labeled diazepam from its receptors, hence acting as diazepam binding inhibitor (DBI)<sup>1</sup>. There are two benzodiazepine receptors, the peripheral receptor, a mitochondrion-located translocator protein (TSPO), and a central receptor, which is the  $\gamma$ -aminobutyric acid (GABA) A receptor (GABA<sub>A</sub>R), the major inhibitory neurotransmitter receptor in the central nervous system. Full length ACBP/DBI displaces diazepam from both TSPO and GABA<sub>A</sub>R. In the central nervous system, ACBP/DBI produced by astrocytes and other cell types can be subjected to endoproteolytic cleavage to generate neuropeptides such as triakontatetrapeptide (residues 17–50) that acts as a selective ligand of TSPO and octadecaneuropeptide (residues 33–50) that acts as an allosteric modulator of GABA<sub>A</sub>R activity<sup>1</sup>.

Recently, we reported that ACBP/DBI plasma concentration is abnormally high in obese individuals, correlating with the fact that the periumbilical fat from obese persons expresses high levels of ACBP/DBI mRNA that diminish upon dietary intervention<sup>2</sup>. Similarly, in mice, obesity induced by a high-fat diet or a genetic deficiency of leptin results into increased expression of ACBP/DBI mRNA and protein in the liver and in adipose tissue, accompanied by an increase in circulating ACBP/DBI protein levels<sup>2</sup>. Conversely, anorexia nervosa is associated with a reduction in ACBP/DBI plasma level<sup>2,3</sup>. A prior study had shown that in patients with acute inflammatory disease, ACBP/DBI plasma levels increase, positively correlating with tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) levels<sup>4</sup>. Intriguingly, obesity is coupled to a state of chronic inflammation in which TNF $\alpha$  is elevated, contributing to the development of insulin-resistant (type-2) diabetes<sup>5,6</sup>. This points to a relationship between metabolic inflammation and the elevation of mediators such as ACBP/DBI and TNF $\alpha$ .

Experiments in mice revealed that intravenous (i.v.) injection of ACBP/DBI protein or transgenic expression of ACBP/DBI in the liver caused hyperphagy and weight gain. Conversely, neutralization of ACBP/DBI by an inducible whole-body knockout or intraperitoneal (i.p.) injection of neutralizing antibodies had anorexigenic effects, reducing food intake and lipo-anabolic reactions, while increasing lipo-catabolism (such as lipolysis and fatty acid oxidation), thus reducing weight gain in the context of a high-fat diet or leptin deficiency or enhancing weight loss upon a switch from a high-fat diet to a normal diet<sup>2</sup>. These findings, combined with the fact that ACBP/DBI, an evolutionarily ancient gene/protein, can stimulate sporulation in unicellular yeast species<sup>7,8</sup> and in slime molds<sup>9</sup>, pharyngeal pumping in nematodes<sup>8</sup>, and mouse hook movement (the equivalent of mastication) in flies<sup>10</sup> let us to postulate that ACBP/DBI is the elusive phylogenetically conserved appetite stimulator or “hunger factor”<sup>11,12</sup>.

Eating disorders such as anorexia nervosa and morbid obesity are metabolic diseases with a psychiatric component. Importantly, prototypic psychiatric diseases including treatment-resistant depression and severe schizophrenia are coupled to major derangements in appetite and body weight and often lead to a state of metabolic syndrome that negatively affects life expectancy<sup>13–15</sup>. Obviously, the GABAergic system composed by GABA and its receptors plays a major role in the central nervous system<sup>16,17</sup> as well as in the regulation of metabolism<sup>18</sup> and inflammation<sup>19</sup>.

Intrigued by these premises, we decided to investigate the possible impact of ACBP/DBI on psychiatric conditions. For this, we addressed the questions as to whether ACBP/DBI stimulates appetite through its binding to acyl CoA or an action on GABA<sub>A</sub>R and whether ACBP/DBI affects the behavior of mice upon its artificial elevation or neutralization in peripheral tissues. We also measured ACBP/DBI concentration in the plasma of psychiatric patients to understand its potential impact on mental vs. metabolic disease.

## Materials and methods

### Mouse experiments

Eight- to ten-week-old male C57BL/6 mice, Wild-type (WT, Envigo, Gannat, France and Janvier, Le Genest-Saint-Islen, France), B6.Cg-Lep<sup>ob</sup>/J *ob/ob* mice, S/B6.V-LEP+/Ob (JAX<sup>™</sup> Mice Strain, Charles River Laboratory, Lentilly, France) or Gabrg2<sup>tm1<sup>Wul</sup></sup>/J, containing the point mutation F77I in the gamma-aminobutyric acid (GABA) A receptor  $\gamma$ 2 subunit<sup>20</sup> (JAX<sup>™</sup> Mice Strain, Charles River Laboratory, Lentilly, France) were bred and maintained according to the FELASA guidelines and local guidelines from the Animal Experimental Ethics Committee (#04447.02, #2315-2015101617138161v1, #8530-2017011216394941v2, #10862-2017080217568517v3, #25032, 19144-2018050412 55279v2, France).

### Treatments

Mice were housed in a temperature-controlled environment with 12 h light/dark cycles and received normal diet and water ad libitum. Mice were subjected to 24 h starvation (Unfed), injected intraperitoneally or intravenously and cumulative food intake was analyzed. The mAb 7A antibody against ACBP/DBI or the isotype IgG2a control were used in vivo (5  $\mu$ g/g body weight (BW), i.p. in total volume 200  $\mu$ L) (Fred Hutch Antibody Technology, Seattle, WA, USA). Recombinant mouse ACBP/DBI (i.v., in total volume of 200  $\mu$ L, 0.5 mg/kg BW) (recACBP/DBI, from Institute of Psychiatry and Neuroscience of Paris, France) or the vehicle control (phosphate-buffered saline) were used in vivo. Moreover, two mutant forms of mouse recombinant ACBP/DBI were used in which two conserved residues were substituted (Y29F and K33A), reducing the affinity of ACBP/DBI for

the acyl-CoAs<sup>21</sup>. Recombinant mouse Ghrelin (purchased by Merck Millipore) was administered by i.p. injection at 10 µg/25 g BW.

#### Food intake analysis

Food intake was monitored as previously described<sup>2</sup>. In brief, food was removed 2 h prior to experimentation followed by individual housing and acclimatization in individual cages. Different treatments were administered and the accumulated food intake was monitored.

#### Light-to-dark transition test (D/LT)

Test based on the innate aversion of rodents to brightly illuminated areas and on their spontaneous exploratory behavior in response to the stressor that light represents<sup>22</sup>. The test apparatus consists of a dark, safe compartment and an illuminated, aversive one (43 × 43 cm chamber). The lit compartment was brightly illuminated with an 8 W fluorescent tube (1000 lx). Naive mice were placed individually in the testing chamber in the middle of the dark area facing away from the doorway to the light compartment. Mice were tested for 10 min, and four parameters were recorded: time spent in the lit compartment, the number of transitions between compartments, the speed of the mice and the distance spent in the lit compartment indices of anxiety-related behavior and exploratory activity. Behavior was scored using an infrared light beam activity monitor using actiMot2 Software (PhenoMaster Software, TSE) and it was statistically analyzed using Prism program.

#### Open-field test (OFT)

Test takes advantage of the aversion of rodents to brightly lit areas<sup>22</sup>. Each mouse is placed in the center of the OFT chamber (43 × 43 cm chamber) and allowed to explore for 30 min. Mice were monitored throughout each test session by infrared light beam activity monitor using actiMot2 Software (PhenoMaster Software, TSE). The overall motor activity was quantified as the total distance travelled (ambulation). Anxiety was quantified by measuring the time and distance spent in the center versus periphery of the open-field chamber. Behavior was scored using an infrared light beam activity monitor using actiMot2 Software (PhenoMaster Software, TSE) and it was statistically analyzed using Prism program.

#### Forced swim test (FST)

Test based on the observation that rodents, after initial escape-oriented movements, develop an immobile posture when placed in an inescapable stressful situation<sup>23</sup>. Each mouse is placed in a cylinder (height: 25 cm and diameter: 10 cm) filled with water (23–25 °C). Mice were tested for 5 min, and the time spent immobile (behavioral despair) was quantified.

#### ACBP/DBI detection in human plasma samples

Plasma ACBP/DBI levels were measured in two different cohorts of bipolar and schizophrenic patients, by means of the KA0532 ACBP (Human) ELISA kit. The subjects ( $n = 271$ ) were participants of the FACE-BD and FACE-SZ studies<sup>13–15</sup>. Dyslipidaemia and type 2 diabetes were extracted from patient's medical history. Hypertension was defined as systolic blood pressure  $\geq 140$  and/or diastolic blood pressure  $\geq 90$  mmHg. Abdominal obesity was defined as waist circumference  $\geq 94$  cm or 37 in. (male) or  $\geq 80$  cm or 31.5 in. (female). Metabolic syndrome was defined according to the International Diabetes Foundation definition<sup>24</sup>.

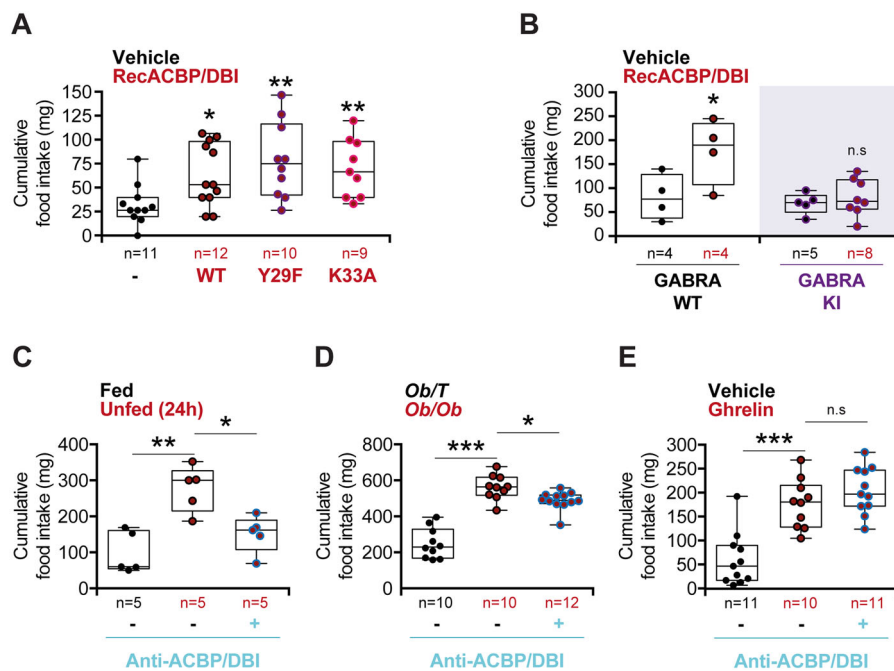
#### Statistical analysis

Data are reported as Box and whisker plots (mean, first and third quartiles, and maximum and minimum values). The number of independent data points ( $n$ ) is indicated in the corresponding graphs or in the legends. For statistical analyses,  $p$  values were calculated by two-way ANOVA, one-way ANOVA with Tukey's multiple comparisons test or two-tailed unpaired Student's  $t$  test as indicated (Prism version 7, GraphPad Software). Differences were considered statistically significant when  $p$  values  $*(p < 0.05)$ ,  $** (p < 0.01)$ ,  $*** (p < 0.001)$ , and n.s. = not significant ( $p > 0.05$ ). For the analysis of human samples, means ( $\pm$  standard deviation or standard error of the mean) were compared with two-tailed unpaired Student's  $t$  test and Pearson's correlation coefficients with their 95% confidence interval were calculated. A generalized linear model was constructed to calculate odds ratios between ACBP/DBI (per 1 ng/mL increase) and categorical metabolic variables in a univariate model and in a multivariate model incorporating body mass index (BMI).

## Results

#### Appetite stimulation by ACBP/DBI in mice through an action on GABA<sub>A</sub> receptors

As indicated by its dual name, ACBP/DBI has two fundamentally distinct functions, as a protein that binds acyl coenzyme A (CoA) and as a protein that binds to GABA<sub>A</sub>R. The interaction with acyl-CoA is reduced by 3 orders of magnitude upon mutation of tyrosine residue 29 to phenylalanine, Y29F, or mutation of lysine residue 33 to alanine, K33A (Supplementary Fig. S1a)<sup>21,25</sup>. I.v. injection of such mutated Y29F or K33A ACBP/DBI recombinant proteins induced a similar hyperphagic response as did the WT protein (Fig. 1a), indicating that appetite stimulation by ACBP/DBI does not rely exclusively on the binding of acyl-CoA-related metabolites. The action of ACBP/DBI on GABA<sub>A</sub> receptor is lost in mice in which the  $\gamma 2$  subunit bears a point mutation substituting the phenylalanine residue 77 to isoleucine, F77I) (Supplementary Fig. S1b)<sup>20,26</sup>. Mice bearing this knockin (KI) mutation



**Fig. 1 Analysis of feeding behavior modulated by ACBP/DBI.** **a** Cumulative food intake was measured after 60 min in WT mice injected with recombinant ACBP/DBI (recACBP/DBI) protein, its mutant forms Y29F or K33A (i.v., 0.5 mg/kg BW) or a vehicle control (**a**), in GABRA WT or GABRA knock-in (KI) mice upon recACBP/DBI i.v. injection (**b**), in WT mice after 24 h of starvation (Unfed) (**c**), in obese *Ob/Ob* or lean *Ob/T* mice (**d**), and in WT mice after Ghrelin injection (i.p., 10  $\mu$ g/25 g BW) (**e**), all of them either alone or in combination with the i.p. injection of an antibody against ACBP/DBI (anti-ACBP/DBI, i.p., 5  $\mu$ g/g BW). Quantitative results are reported as box and whisker plots (mean, first and third quartiles, and maximum and minimum values). For statistical analyses, *p* values were calculated by two-way ANOVA (**b**) or one-way ANOVA with Tukey's multiple comparisons test (**a**, **c–e**). Differences were considered statistically significant when *p* values \* ( $p < 0.05$ ), \*\* ( $p < 0.01$ ), \*\*\* ( $p < 0.001$ ) and n.s. not significant ( $p > 0.05$ ).

failed to mount a hyperphagic response upon injection of WT ACBP/DBI in conditions in which age- and sex-matched WT control mice did increase their food intake (Fig. 1b), indicating that ACBP/DBI indeed acts on GABA<sub>A</sub> receptors to stimulate appetite.

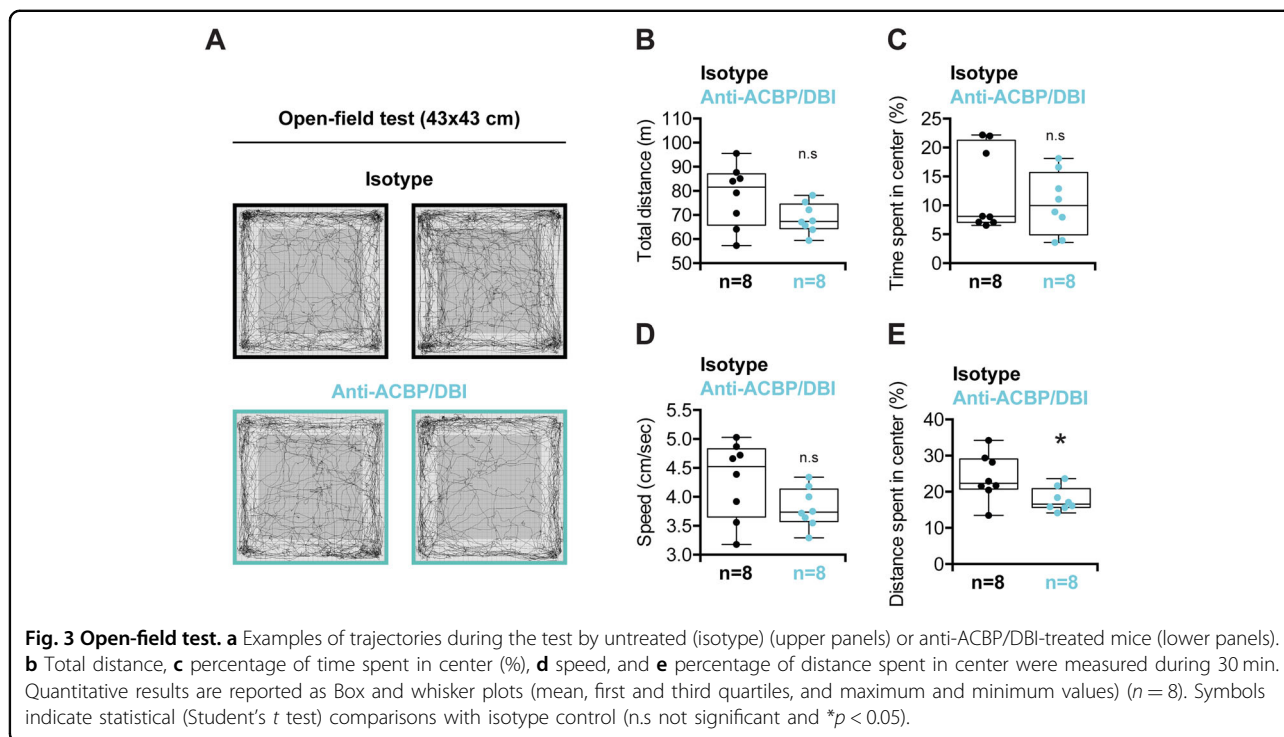
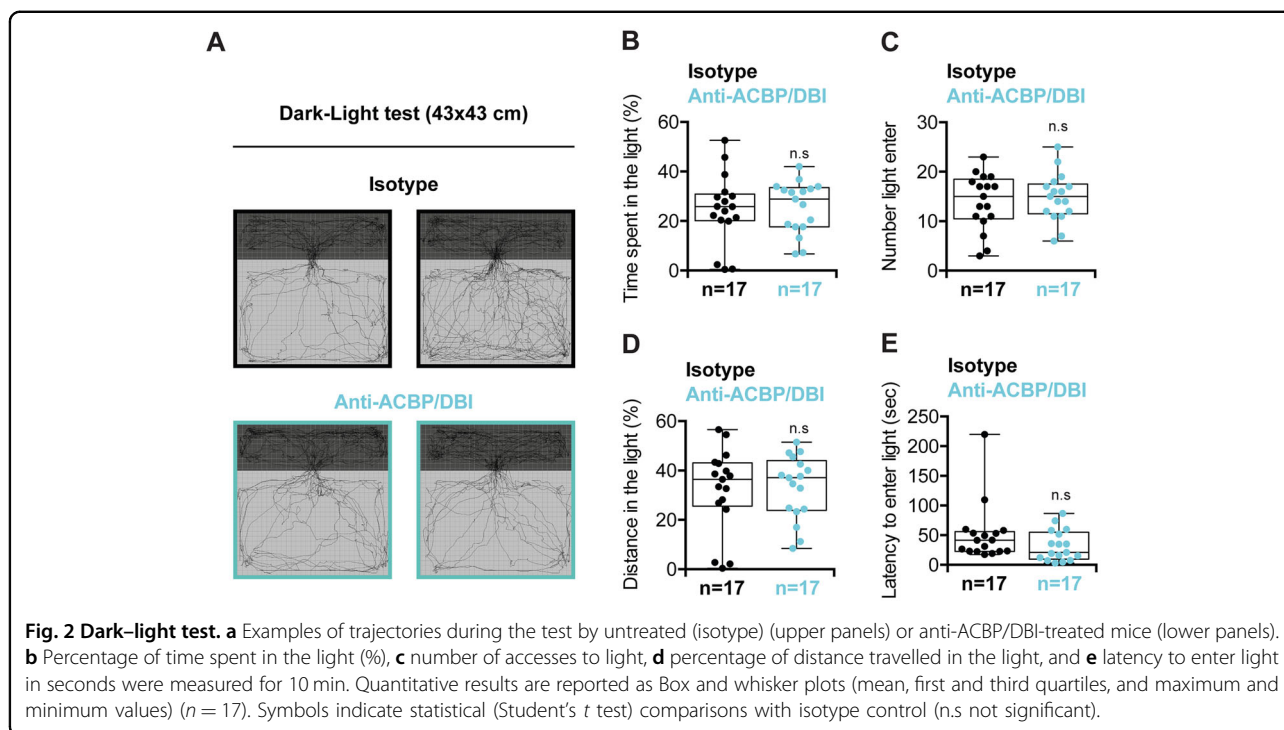
Of note, i.p. injection of a neutralizing ACBP/DBI-specific monoclonal antibody (mAb) was able to inhibit food intake in mice that had been rendered hyperphagic (Supplementary Fig. S1c) by a 24-h starvation period (Fig. 1c). Similarly, anti-ACBP/DBI mAb reduced food intake in mice homozygous for the *Lep<sup>ob</sup>* mutation (often referred to as *ob/ob* mice) that are rendered hyperphagic due to a mutation in the gene encoding for the appetite inhibitor leptin (Fig. 1d). In contrast, ACBP/DBI neutralization was unable to interfere with hyperphagy induced by ghrelin injection (Fig. 1e), indicating that anti-ACBP/DBI mAb has a specific rather than general effect on food intake. Thus, the possibility that anti-ACBP/DBI mAb would simply induce a general lethargy that compromises food intake can be excluded.

#### Effects of ACBP/DBI on the behavior of mice

Pharmacological agents acting on GABA<sub>A</sub> receptors (which include anesthetics, barbiturates, benzodiazepines,

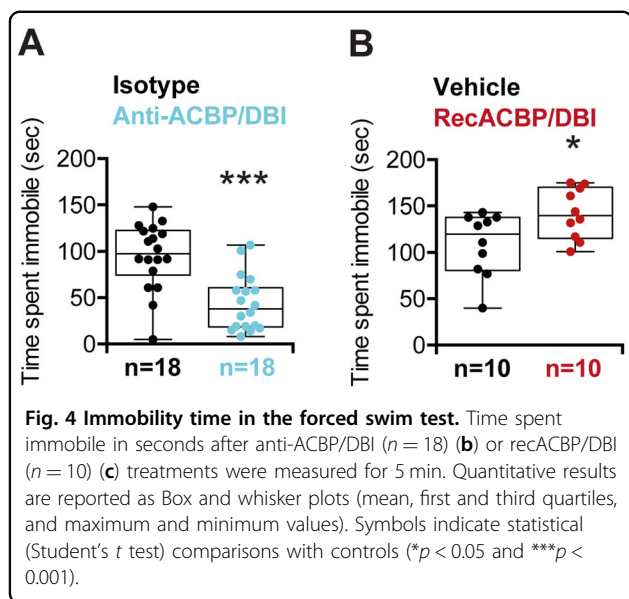
and zolpidem) have major effects on human behavior<sup>16,27</sup>, and several appetite-inhibitory agents have been rejected or withdrawn by either the FDA or EMA (or both) due to an increase in depression and suicide<sup>28,29</sup>, prompting us to assess the behavioral effects of ACBP/DBI neutralization in mouse models. In the light–dark box test, which measures unconditioned anxiety and that accurately reflects the anxiolytic effects of benzodiazepines<sup>30,31</sup>, mice receiving the neutralizing anti-ACBP/DBI antibody exhibited a similar behavior as control mice injected with an isotype control antibody (Fig. 2). Similarly, ACBP/DBI injection had no impact on this behavioral test (Supplementary Fig. S2). The open-field test, which measures general locomotor activity levels, anxiety, and willingness to explore, is known to be sensitive to benzodiazepines<sup>32–34</sup>. ACBP/DBI neutralization had no major effects on the open-field test, except for a longer distance spent in the center of the box, suggesting a mild anxiolytic activity for the anti-ACBP/DBI antibody (Fig. 3). However, recombinant ACBP/DBI did not affect the open-field test (Supplementary Fig. S3).

Next, we took advantage of the Porsolt forced swim test (also called “behavioral despair test”) (Supplementary Fig. S4a), which is used to detect a depression-like behavior, reflected by a premature switch from swimming to



immobile floating<sup>23</sup>. Benzodiazepines are well known to enhance the immobile behavior in this test in a dose-dependent fashion<sup>35,36</sup>. Of note, the anti-ACBP/DBI antibody reduced the immobile behavior of mice (Fig. 4a), while

injection of recombinant ACBP/DBI protein enhanced the floating behavior (Fig. 4b), in line with the interpretation that ACBP/DBI neutralization has an antidepressant effect. Of note, the effects of ACBP/DBI on depression were lost



when the protein was mutated to suppress its acyl CoA binding ability (Supplementary Fig. S4b).

#### Plasma ACBP/DBI levels in patients diagnosed with severe depression or schizophrenia

In the next step, we measured the circulating ACBP/DBI concentration in patients with bipolar disorder or schizophrenia. No difference in the plasma ACBP/DBI levels was detectable between depressive and schizophrenic patients (Fig. 5a, Supplementary Fig. S5a). In both groups, ACBP/DBI levels similarly correlated with the BMI, body weight or waist circumference (Supplementary Fig. S5d), as previously described for a series of patients with eating disorders including anorexia nervosa and morbid obesity<sup>2</sup>. ACBP/DBI concentrations did not correlate with subsequent weight variations, meaning that there were no significant variations in ACBP/DBI concentration between patients with minor weight oscillations (by <5%), significant weight loss (≥5%) or weight gain (≥5%) within the 6 months following the ACBP/DBI measurement (Fig. 5b, Supplementary Fig. S5b).

Nonetheless, patients with metabolic syndrome tended to exhibit a higher ACBP/DBI concentration than patients without metabolic syndrome (*p* = 0.097) (Fig. 6a, Supplementary Fig. S5c). However, multivariate analysis indicated that ACBP/DBI was not associated with metabolic syndrome, independently of BMI. In contrast, variations in ACBP/DBI plasma levels were associated with dyslipidemia (*p* = 0.003) (but not with diabetes nor arterial hypertension), and this association was independent of BMI (*p* = 0.055) (Fig. 6b, c).

Altogether, these results suggest that ACBP/DBI levels are well correlated with BMI, irrespective of their

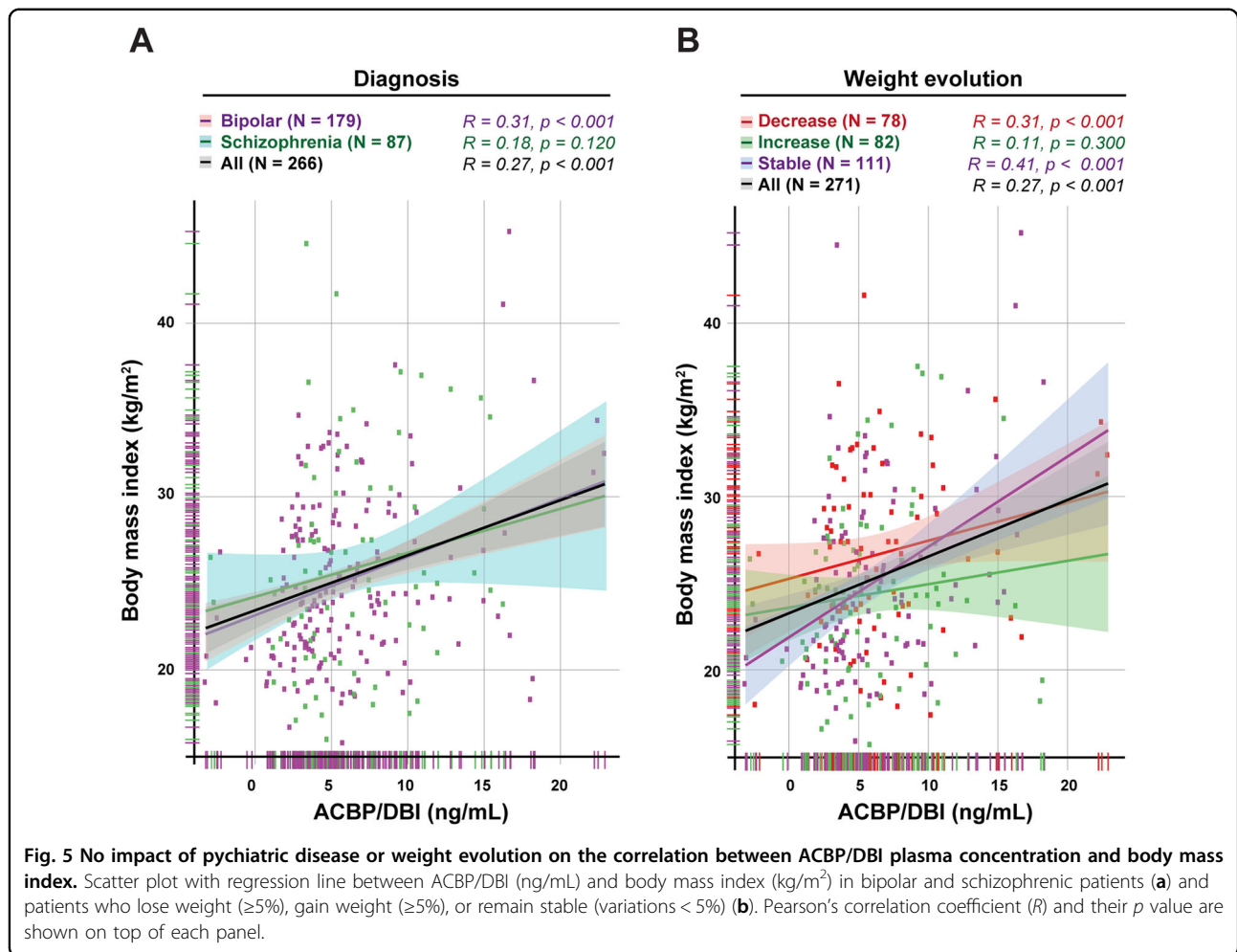
psychiatric diagnosis, do not predict later changes in BMI, yet are associated with dyslipidemia.

#### Discussion

ACBP/DBI is a phylogenetically ancient protein that stimulates appetite and lipo-anabolism in animals, ranging from nematodes and insects to rodents<sup>8,12</sup>. It is also elevated in human obesity but reduced in anorexia nervosa<sup>2</sup>. For this reason, neutralization of ACBP/DBI by suitable antibodies might constitute a valid strategy for combating obesity and its co-morbidities<sup>12,37,38</sup>. Given the fact that several anorexigenic drugs have been withdrawn from the clinics due to their psychiatric side effects<sup>27,28</sup>, we evaluated the behavioral effect of ACBP/DBI and ACBP/DBI neutralizing antibodies in rodent models and attempted to establish a correlation between mental disease and circulating ACBP/DBI concentrations in psychiatric patients.

Mouse experiments detailed in this paper revealed that the orexigenic effect of systemically (i.v.) injected ACBP/DBI protein did not rely on its interaction with acyl-CoA but apparently involved an action on GABA<sub>A</sub>R, alerting about the possibility that ACBP/DBI might indeed affect GABA-regulated mood control. However, at odds with this possibility, neither the systemic (i.v.) injection of ACBP/DBI nor the systemic (i.p.) administration of a neutralizing ACBP/DBI antibody did affect the behavior of mice in the light–dark test and in the open-field tests. In contrast, ACBP/DBI injection caused a “depression-like” behavior in the forced swim test, meaning that the mice ceased active swimming and switched toward passive floating earlier than sham-injected mice. Conversely, neutralization of ACBP/DBI resulted into an “anti-depressant” effect, prolonging the active combat of mice for survival. The effects of ACBP/DBI on depression depend on its acyl CoA binding ability, while induction of hyperphagy by ACBP/DBI did not require acyl CoA binding. These discrepant findings underscore that (some of) the metabolic and mood-modulating effect of ACBP/DBI can be uncoupled from each other.

Mice that are constitutively knockout for ACBP/DBI (meaning that the gene is even expressed during embryogenesis) exhibit a stereotyped self-grooming behavior, reduced social interest, but normal social recognition<sup>39</sup>, pointing to a minor behavioral phenotype. In contrast, we have not noted any evident changes in mouse behavior after the inducible knockout of ACBP/DBI in adult mice<sup>2</sup>, suggesting that these effects might be linked to neurodevelopment. Intracerebroventricular administration of recombinant ACBP/DBI or that of ACBP/DBI-derived neuropeptides induces proconflict behavior<sup>40</sup>, stimulates anxiety<sup>41</sup>, and reduces food intake<sup>42</sup>, causing a loss in bodyweight in long-term experiments<sup>43</sup>. When micro-injected into the swallowing pattern generator located in the nucleus tractus solitarius, the octadecaneuropeptide



derived from ACBP/DBI inhibits the swallowing reflex<sup>42</sup>. Of note, the anorexigenic effects of octadecaneuropeptide do not depend on an action on TSPO or GABA<sub>A</sub>R but rather on a G protein coupled receptor<sup>44,45</sup>. Thus, the central (i.c.v.) injection of ACBP/DBI causes GABA<sub>A</sub>R-independent anorexigenic effects that are diametrically opposed to the GABA<sub>A</sub>R-dependent orexigenic effects observed after its peripheral (i.v.) administration<sup>2</sup>. Of note, it appears plausible that i.v. administered ACBP/DBI mediates its effects through an action on peripheral metabolism, causing a hypoglycemic response that then activates orexigenic neurons in the hypothalamus. Indeed, artificial maintenance of glucose concentrations by a glucose clamp prevents the activation of such orexigenic neurons as well as the hyperphagic response of mice<sup>2</sup>.

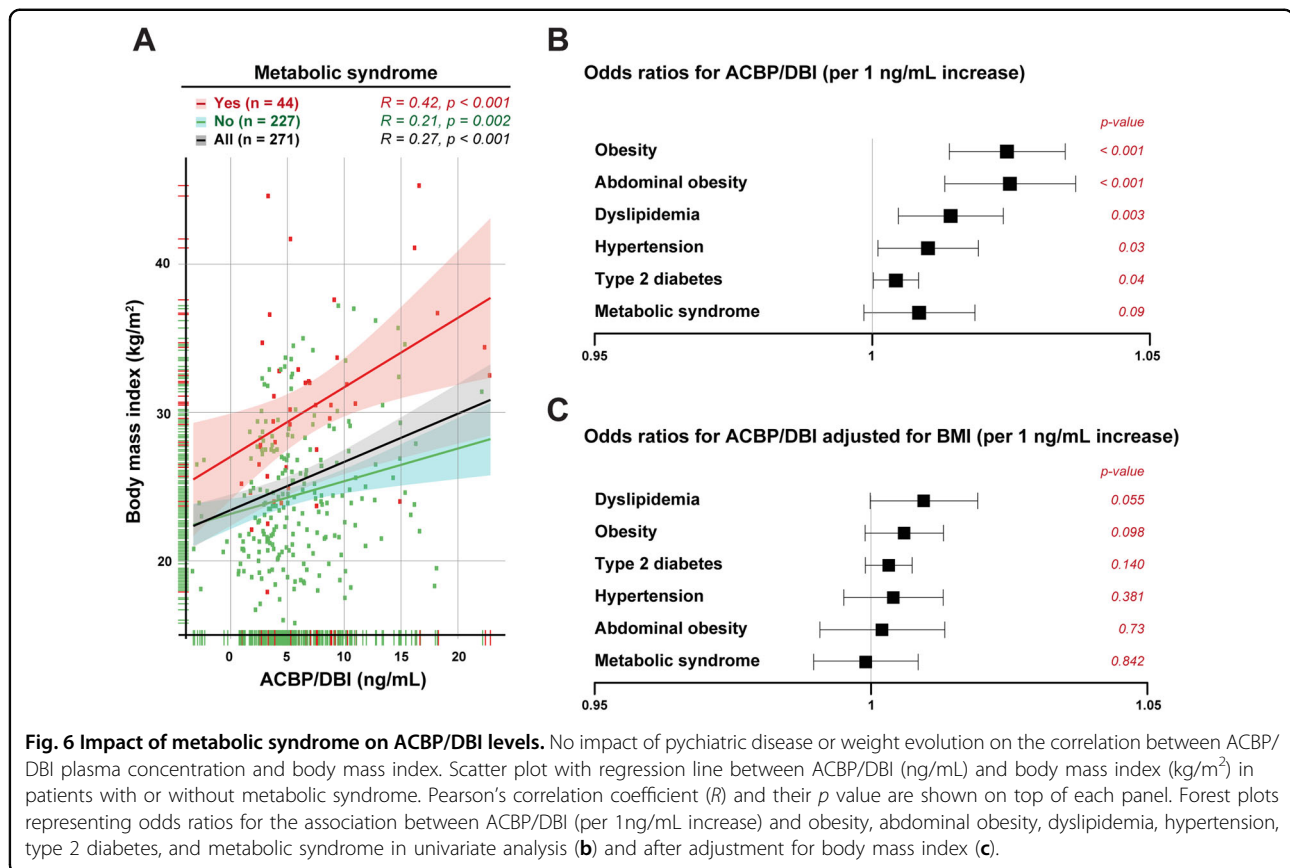
Obviously, it will be interesting to investigate the impact of ACBP/DBI on the expression of its receptors (in particular GABA<sub>A</sub>R subunits and the mitochondrial TSPO protein), the expression level of other neuroendocrine factors, as well as bioenergetic parameters in multiple different peripheral and central nervous tissue to

understand the full range of its physiological effects. Thus a single-cell multi-omics approach (including but not limited to transcriptomics, proteomics and metabolomics) should be envisaged in the future to explore the effects of ACBP/DBI in further detail.

In line with the idea that the peripheral pool of ACBP/DBI has little impact on mental operations, we did not observe any difference between schizophrenic and bipolar patients with respect to their plasma ACBP/DBI concentrations, which however strongly correlated with BMI in both groups. The levels of ACBP/DBI concentrations measured at diagnosis did not allow predicting the subsequent trajectory of BMI (gain, loss, or stability) and rather correlated with the actual state of the BMI. However, a high ACBP/DBI plasma concentration constitutes a risk factor for dyslipidemia, independently from BMI, as indicated by multivariate analysis. This result strongly pleads in favor of a role of ACBP/DBI in metabolism that is more important than its putative role in mental disease.

As a final note, it appears important that ACBP/DBI neutralization, which might constitute a novel treatment





for obesity and its comorbidities such as type-2 diabetes and non-alcoholic steatohepatitis, has no unwarranted (depression- or anxiety-inducing) effects on mice. This preclinical finding may facilitate the development of a novel type of antiobesity medication that targets ACBP/DBI or its interaction with peripheral GABA<sub>A</sub>R.

#### Acknowledgements

The authors thank Maxime Perez for establishing contacts among dispersed investigators. The authors thank the CRC Core Facilities for the technical support. G.K. is supported by the Ligue contre le Cancer (équipe labellisée); Agence Nationale de la Recherche (ANR)—Projets blancs; ANR under the frame of E-Rare-2, the ERA-Net for Research on Rare Diseases; AMMICA US23/CNRS UMS3655; Association pour la recherche sur le cancer (ARC); Association "Le Cancer du Sein, Parlons-en!"; Cancéropôle Ile-de-France; Chancellerie des universités de Paris (Legs Poix), Fondation pour la Recherche Médicale (FRM); a donation by Elior; European Research Area Network on Cardiovascular Diseases (ERA-CVD, MINOTAUR); Gustave Roussy Odyssey, the European Union Horizon 2020 Project Oncobiome; Fondation Carrefour; High-end Foreign Expert Program in China (GDW20171100085), Institut National du Cancer (INCa); Inserm (HTE); Institut Universitaire de France; LeDucq Foundation; the LabEx Immuno-Oncology (ANR-18-IDEX-0001); the RHU Torino Lumière; the Seerave Foundation; the SIRIC Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (SOCRATE); and the SIRIC Cancer Research and Personalized Medicine (CARPEM). J.M.B.-S.P. is supported by a Ramon y Cajal contract funded by the Spanish Government.

#### FACEGroups members List of FondaMental Advanced Center of Expertise (FACE-BD) collaborators

FACE-BD Clinical Coordinating Center (Fondation FondaMental); B. Etain, C. Henry, E. Olié, M. Leboyer, E. Haffen and PM Llorca; FACE-BD Data Coordinating

Center (Fondation FondaMental); V. Barteau, S. Bensalem, O. Godin, H. Laouamri, and K. Souryis; FACE-BD Clinical Sites and Principal Collaborators in France; AP-HP, DHU PePSY, Pôle de Psychiatrie et d'Addictologie des Hôpitaux Universitaires H Mondor, Créteil; S. Hotier, A. Pelletier, N. Drancourt, JP. Sanchez, E. Saliou, C. Hebbache, J. Petrucci, L. Willaume and E. Bourdin; AP-HP, GH Saint-Louis-Lariboisière-Fernand Widal, Pôle Neurosciences, Paris; F. Bellivier, M. Carminati, B. Etain, J. Maruani, E. Marlinge, J. Meheust and S. Yeim; Hôpital C. Perrens, Centre Expert Trouble Bipolaire, Service de Psychiatrie Adulte, Pôle 3-4-7, Bordeaux; B. Antoniol, A. Desage, S. Gard, A. Jutant, K. Mbailara, I. Minois, and L. Zanouy; Département d'Urgence et Post Urgence Psychiatrique, CHRU Montpellier, Montpellier; C. Abettan, L. Bardin, A. Cazals, P. Courtet, B. Deffinis, D. Ducasse, M. Gachet, A. Henrion, E. Martinerie, F. Molière, B. Noisette, E. Olié and G. Tarquini; Pôle de Psychiatrie, addictologie et pédopsychiatrie, Hôpital Sainte Marguerite, Marseille; R. Belzeaux, N. Correard, F. Groppi, A. Lefrere, L. Lescalier, E. Moreau, J. Pastol, M. Rebattu, B. Roux and N. Viglianesi; Service de Psychiatrie et Psychologie Clinique, CHU de Nancy, Hôpitaux de Brabois, Vandoeuvre Les Nancy; R. Cohen, Raymond Schwan, J.P. Kahn, M. Milazzo, and O. Wajsbrot-Elgrabli; Service Universitaire de Psychiatrie, CHU de Grenoble et des Alpes, Grenoble; T. Bougerol, B. Fredembach, A. Suisse, B. Halili, Z. Gaoua, and M. Polosan; Centre Hospitalier de Versailles, Service Universitaire de Psychiatrie d'adultes, Le Chesnay; A.M. Galliot, I. Grévin, A.S. Cannavo, N. Kayser, C. Passerieux, and P. Roux; Service de Psychiatrie, Centre Hospitalier Princesse Grace, Monaco; V. Aubin, I. Cussac, M.A Dupont, J. Loftus, and I. Medecin; AHPH, Département de Psychiatrie, Hôpital Louis Mourier, Colombes, France; C. Dubertret, N. Mazer, C. Portalier.

#### List of FondaMental Advanced Center of Expertise (FACE-SZ) collaborators

FACE-SZ Clinical Coordinating Center (Fondation FondaMental); F. Berna, E. Haffen, M. Leboyer, PM Llorca and F. Schürhoff; FACE-SZ Data Coordinating Center (Fondation FondaMental); V. Barteau, S. Bensalem, O. Godin, H.

Laouamri and K. Souryis; FACE-SZ Clinical Sites and Principal Collaborators in France; AP-HP, INSERM U955, Translational Psychiatry Team, DHU Pe-PSY, Centre Expert Schizophrénie, Pôle de Psychiatrie et d'Addictologie des Hôpitaux Universitaires Henri Mondor, Paris Est University, 40 rue de Mesly, 94000 Créteil, France: N. Coulon, M. Leboyer, I. Offerlin-Meyer, B. Pignon, F. Schürhoff and A. Szöke; Department of Adult Psychiatry, Charles Perrens Hospital, F-33076 Bordeaux, France; Laboratory of Nutrition and Integrative Neurobiology (UMR INRA 1286), University of Bordeaux, France: B. Aouizerate; Department of Adult Psychiatry, Charles Perrens Hospital, F-33076 Bordeaux; University of Bordeaux, CNRS UMR 5287-INCIA, Bordeaux, France: A. Deloge, D. Misdráhi and E. Vilà; CHU Clermont-Ferrand, Department of Psychiatry (service de psychiatrie B), University of Clermont Auvergne, Clermont-Ferrand, France: O. Blanc, I. Chéreau, H. Denizot, JM. Honciuc, D. Laccelle, PM. Llorca and S. Pires; AP-HP, Department of Psychiatry, Louis Mourier Hospital, Colombes, Inserm UMR1266, Institute of Psychiatry and Neurosciences of Paris, University Paris Descartes, Université Paris Diderot, Sorbonne Paris Cité, Faculté de médecine, France: C. Dubertret, J. Mallet, and C. Portalier; Psychosocial Rehabilitation Reference Center, Alpes Isère Hospital, Grenoble, France: J. Dubreucq, C. Fluttaz, F. Gabayet and C. Roman; University Claude Bernard Lyon 1, Le Vinatier Hospital Pole Est BP 300 39 - 95 bd Pinel - 69678 Bron Cedex, France: G. Chesnoy-Servanin, T. D'Amato, JM. Dorey, R. Rey and A. Vehier; Department of Psychiatry (AP-HM), Sainte-Marguerite University Hospital, Marseille, France: C. Lançon, C. Faget, E. Metaire, P. Peri and F. Vaillant; AP-HM, la Conception Hospital, Aix-Marseille Univ, School of medicine - La Timone Medical Campus, EA 3279: CEReSS - Health Service Research: L. Boyer and G. Fond; Strasbourg University Hospital, University of Strasbourg, INSERM U1114, Federation of Translational Psychiatry, Strasbourg, France: F. Berna, P. Vidailhet and A. Zinetti-Bertschy; University Department of Adult Psychiatry, La Colombiere Hospital, CHU Montpellier, University of Montpellier 1, Inserm 1061, Montpellier, France: D. Capdevielle and H. Yazbek; Department of Adult Psychiatry, Versailles Hospital, Le Chesnay, France; HandiRESP and Quality of Life Center, 27 Boulevard Jean Moulin, 13005 Marseille, France Laboratory, EA4047, UFR Health Sciences Simone Veil, Université de Versailles Saint-Quentin-En-Yvelines, Montigny-le-Bretonneux, France: S. Esselin, M. Jarroir, C. Passerieux, and M. Urbach.

#### Author details

<sup>1</sup>Centre de Recherche des Cordeliers, Equipe labellisée par la Ligue Contre le Cancer, Université de Paris, Sorbonne Université, Inserm U1138, Paris, France. <sup>2</sup>Metabolomics and Cell Biology Platforms, Institut Gustave Roussy, Villejuif, France. <sup>3</sup>Faculté de Médecine, Université de Paris Saclay, Kremlin Bicêtre, France. <sup>4</sup>INSERM U1151, Institut Necker Enfants-Malades (INEM), Université Paris Descartes-Sorbonne-Paris Cité, Paris, France. <sup>5</sup>Cell Biology Group, Department of Experimental and Health Sciences, Pompeu Fabra University (UPF), Barcelona, Spain. <sup>6</sup>Gustave Roussy Comprehensive Cancer Institute, Villejuif, France. <sup>7</sup>Fondation FondaMental, Créteil, France. <sup>8</sup>Université Paris Est Créteil, Inserm U955, IMRB, Laboratoire Neuro-Psychiatrie translationnelle, F-94010 Créteil, France. <sup>9</sup>AP-HP, HU Henri Mondor, Département Medico-Universitaire de Psychiatrie et d'Addictologie (DMU ADAPT), Fédération Hospitalo-Universitaire de Médecine de Precision (FHU IMPACT), F-94010 Créteil, France. <sup>10</sup>Fondation FondaMental Créteil, Créteil, France. <sup>11</sup>Pôle de Psychiatrie, Centre Hospitalier Princesse Grace, Monaco, France. <sup>12</sup>AP-HP, GH Saint-Louis—Lariboisière—Fernand Widal, Pôle Neurosciences Tête et Cou, INSERM UMRS 1144, University Paris Diderot, Paris, France. <sup>13</sup>Pôle de Psychiatrie, Assistance Publique Hôpitaux de Marseille, Marseille, France. <sup>14</sup>INT-UMR7289, CNRS Aix-Marseille Université, Marseille, France. <sup>15</sup>Hôpitaux Universitaires de Strasbourg, Université de Strasbourg, INSERM U1114, Fédération de Médecine Translationnelle de Strasbourg, Strasbourg, France. <sup>16</sup>Service Universitaire de Psychiatrie Adulte, Hôpital la Colombière, CHRU Montpellier, Université Montpellier 1, Inserm 1061, Montpellier, France. <sup>17</sup>Department of Emergency Psychiatry and Acute Care, Lapeyronie Hospital, CHU Montpellier, Montpellier, France. <sup>18</sup>PSNREC, Univ Montpellier, INSERM, CHU Montpellier, Montpellier, France. <sup>19</sup>AP-HP, Groupe Hospitalo-Universitaire Nord, DMU ESPRIT, Service de Psychiatrie et Addictologie, Hôpital Louis Mourier, Colombes, Inserm U1266, Faculté de Médecine, Université de Paris, Paris, France. <sup>20</sup>Centre Référent de Réhabilitation Psychosociale et de Remédiation Cognitive (C3R), CH, Alpes Isère, France. <sup>21</sup>INSERM U1028, CNRS UMR5292, Centre de Recherche en Neurosciences de Lyon, Université Claude Bernard Lyon 1, Equipe PSYR2, Centre Hospitalier Le Vinatier, Pole Est, 69678 Bron Cedex, France. <sup>22</sup>AP-HM, Aix-Marseille University, School of Medicine—La Timone Medical Campus, EA

3279, Marseille, France. <sup>23</sup>EReSS—Health Service Research and Quality of Life Center, 13005 Marseille, France. <sup>24</sup>Centre Expert Troubles Bipolaires, Service de Psychiatrie Adulte, Hôpital Charles-Perrens, Bordeaux, France. <sup>25</sup>CHU Clermont-Ferrand, Department of Psychiatry, University of Clermont Auvergne, Clermont-Ferrand, France. <sup>26</sup>Service Universitaire de Psychiatrie d'Adultes, Centre Hospitalier de Versailles, Le Chesnay, Université Paris-Saclay, UVSQ, Inserm, CESP, Team "DevPsy", 94807 Villejuif, France. <sup>27</sup>Université Grenoble Alpes, CHU de Grenoble et des Alpes, Grenoble Institut des Neurosciences (GIN) Inserm U 1216, Grenoble, France. <sup>28</sup>Université de Lorraine, CHRU de Nancy et Pôle de Psychiatrie et Psychologie Clinique, Centre Psychothérapique de Nancy, Nancy, France. <sup>29</sup>Université de Paris, BFA, UMR 8251, CNRS, Paris, France. <sup>30</sup>University Complutense de Madrid. Faculty of Medicine. Department of Physiology, Madrid, Spain. <sup>31</sup>Pôle de Biologie, Hôpital Européen Georges Pompidou, AP-HP, Paris, France. <sup>32</sup>Suzhou Institute for Systems Medicine, Chinese Academy of Medical Sciences, Suzhou, China. <sup>33</sup>Karolinska Institute, Department of Women's and Children's Health, Karolinska University Hospital, Stockholm, Sweden

#### Competing interests

G.K. and J.M.B.-S.P. filed a patent application dealing with targeting the ACBP/DBI system in anorexia, obesity, and co-morbidities. G.K. filed additional patent applications dealing with caloric restriction mimetics (autophagy inducers) for the treatment of aging, age-related diseases, cancer, obesity, and co-morbidities. G.K. is a scientific co-founder of Samsara Therapeutics and Therafast Bio.

#### Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Supplementary Information** accompanies this paper at (<https://doi.org/10.1038/s41419-020-2716-5>).

Received: 21 April 2020 Accepted: 23 June 2020

Published online: 06 July 2020

#### References

1. Tonon, M.-C. et al. Endozepines and their receptors: structure, functions and pathophysiological significance. *Pharmacol. Ther.* **208**, 107386 (2020).
2. Bravo-San Pedro, J. M. et al. Acyl-CoA-binding protein is a lipogenic factor that triggers food intake and obesity. *Cell Metab.* **30**, 1171 (2019).
3. Conti, E. et al. Reduced fasting plasma levels of diazepam-binding inhibitor in adolescents with anorexia nervosa. *Int. J. Eat. Disord.* **46**, 626–629 (2013).
4. Clavier, T. et al. Increased plasma levels of endozepines, endogenous ligands of benzodiazepine receptors, during systemic inflammation: a prospective observational study. *Crit. Care Lond. Engl.* **18**, 633 (2014).
5. Dandona, P. et al. Tumor necrosis factor- $\alpha$  in sera of obese patients: fall with weight loss. *J. Clin. Endocrinol. Metab.* **83**, 2907–2910 (1998).
6. Hotamisligil, G. S. Inflammation, metaflammation and immunometabolic disorders. *Nature* **542**, 177–185 (2017).
7. Manjithaya, R., Anjard, C., Loomis, W. F. & Subramani, S. Unconventional secretion of *Pichia pastoris* Acb1 is dependent on GRASP protein, peroxisomal functions, and autophagosome formation. *J. Cell Biol.* **188**, 537–546 (2010).
8. Charmpilas, N. et al. Acyl-CoA-binding protein (ACBP): a phylogenetically conserved appetite stimulator. *Cell Death Dis.* **11**, 7 (2020).
9. Anjard, C. & Loomis, W. F. Peptide signaling during terminal differentiation of *Dictyostelium*. *Proc. Natl Acad. Sci. USA* **102**, 7607–7611 (2005).
10. Ryuda, M. et al. Identification of a novel gene, anorexia, regulating feeding activity via insulin signaling in *Drosophila melanogaster*. *J. Biol. Chem.* **286**, 38417–38426 (2011).
11. Bravo-San Pedro, J. M., Sica, V. & Kroemer, G. The elusive 'hunger protein': an appetite-stimulatory factor that is overabundant in human obesity. *Mol. Cell Oncol.* **6**, e1667193 (2019).
12. Pedro, J. M. B.-S., Sica, V., Madeo, F. & Kroemer, G. Acyl-CoA-binding protein (ACBP): the elusive 'hunger factor' linking autophagy to food intake. *Cell Stress* **3**, 312–318 (2019).

13. Henry, C. et al. Outcomes for bipolar patients assessed in the French expert center network: A 2-year follow-up observational study (FondaMental Advanced Centers of Expertise for Bipolar Disorder [FACE-BD]). *Bipolar Disord.* **19**, 651–660 (2017).
14. Godin, O. et al. Metabolic syndrome, abdominal obesity and hyperuricemia in schizophrenia: Results from the FACE-SZ cohort. *Schizophr. Res.* **168**, 388–394 (2015).
15. Godin, O. et al. Metabolic syndrome and illness severity predict relapse at 1-year follow-up in schizophrenia: the FACE-SZ cohort. *J. Clin. Psychiatry* **79**, 17m12007 (2018).
16. Rudolph, U. & Möhler, H. GABAA receptor subtypes: therapeutic potential in Down syndrome, affective disorders, schizophrenia, and autism. *Annu. Rev. Pharmacol. Toxicol.* **54**, 483–507 (2014).
17. Setiawan, E. et al. Role of translocator protein density, a marker of neuroinflammation, in the brain during major depressive episodes. *JAMA Psychiatry* **72**, 268–275 (2015).
18. Marino, R. A. M. et al. Control of food approach and eating by a GABAergic projection from lateral hypothalamus to dorsal pons. *Proc. Natl Acad. Sci. USA* <https://doi.org/10.1073/pnas.1909340117> (2020).
19. Bhandage, A. K. et al. GABA regulates release of inflammatory cytokines from peripheral blood mononuclear cells and CD4+ T cells and is immunosuppressive in type 1 diabetes. *EBioMedicine* **30**, 283–294 (2018).
20. Wulff, P. et al. From synapse to behavior: rapid modulation of defined neuronal types with engineered GABAA receptors. *Nat. Neurosci.* **10**, 923–929 (2007).
21. Kragelund, B. B. et al. Conserved residues and their role in the structure, function, and stability of acyl-coenzyme A binding protein. *Biochemistry* **38**, 2386–2394 (1999).
22. Fregeac, J. et al. Loss of the neurodevelopmental disease-associated gene miR-146a impairs neural progenitor differentiation and causes learning and memory deficits. *Mol. Autism* **11**, 22 (2020).
23. Krishnan, V. & Nestler, E. J. Animal models of depression: molecular perspectives. *Curr. Top. Behav. Neurosci.* **7**, 121–147 (2011).
24. Alberti, K. G. M. M., Zimmet, P. & Shaw, J. IDF Epidemiology Task Force Consensus Group The metabolic syndrome—a new worldwide definition. *Lancet* **366**, 1059–1062 (2005).
25. Larsen, M. K., Tuck, S., Faergeman, N. J. & Knudsen, J. MAA-1, a novel acyl-CoA-binding protein involved in endosomal vesicle transport in *Caenorhabditis elegans*. *Mol. Biol. Cell* **17**, 4318–4329 (2006).
26. Dumitru, I., Neitz, A., Alfonso, J. & Monyer, H. Diazepam binding inhibitor promotes stem cell expansion controlling environment-dependent neurogenesis. *Neuron* **94**, 125–137.e5 (2017).
27. Rudolph, U. & Knoflach, F. Beyond classical benzodiazepines: novel therapeutic potential of GABAA receptor subtypes. *Nat. Rev. Drug Discov.* **10**, 685–697 (2011).
28. Srivastava, G. & Apovian, C. M. Current pharmacotherapy for obesity. *Nat. Rev. Endocrinol.* **14**, 12–24 (2018).
29. Galaj, E. & Xi, Z.-X. Potential of cannabinoid receptor ligands as treatment for substance use disorders. *CNS Drugs* **33**, 1001–1030 (2019).
30. Belzung, C., Misslin, R., Vogel, E., Dodd, R. H. & Chapouthier, G. Anxiogenic effects of methyl-beta-carboline-3-carboxylate in a light/dark choice situation. *Pharmacol. Biochem. Behav.* **28**, 29–33 (1987).
31. Ennaceur, A. Tests of unconditioned anxiety—pitfalls and disappointments. *Physiol. Behav.* **135**, 55–71 (2014).
32. Engel, O. et al. Imepitoin shows benzodiazepine-like effects in models of anxiety. *Front. Pharm.* **9**, 1225 (2018).
33. Silva, M. T. & Calil, H. M. Screening hallucinogenic drugs: systematic study of three behavioral tests. *Psychopharmacologia* **42**, 163–171 (1975).
34. Stanford, S. C. The Open Field Test: reinventing the wheel. *J. Psychopharmacol.* **21**, 134–135 (2007).
35. Nagatani, T., Yamamoto, T., Sugihara, T. & Ueki, S. The effect of agonists at the GABA-benzodiazepine receptor complex on the duration of immobility of mice in the forced swimming test. *Eur. J. Pharmacol.* **142**, 17–22 (1987).
36. Van der Meersch-Mougeot, V. et al. Benzodiazepines reverse the anti-immobility effect of antidepressants in the forced swimming test in mice. *Neuropharmacology* **32**, 439–446 (1993).
37. Sica, V., Martins, I., Motiño, O., Bravo-San Pedro, J. M. & Kroemer, G. Antibody-mediated neutralization of ACBP/DBI has anorexigenic and lipolytic effects. *Adipocyte* **9**, 116–119 (2020).
38. Kobayashi, H., Maeda, M., Hayata, T. & Kawashima, Y. Clinical study of combination chemotherapy with CDDP, ADM and CPM for ovarian cancer. *Nihon Gan Chiryō Gakkai Shi* **23**, 829–836 (1988).
39. Ujjainwala, A. L., Courtney, C. D., Rhoads, S. G., Rhodes, J. S. & Christian, C. A. Genetic loss of diazepam binding inhibitor in mice impairs social interest. *Genes Brain Behav.* **17**, e12442 (2018).
40. Guidotti, A. et al. Isolation, characterization, and purification to homogeneity of an endogenous polypeptide with agonistic action on benzodiazepine receptors. *Proc. Natl Acad. Sci. USA* **80**, 3531–3535 (1983).
41. De Mateos-Verchere, J. G., Leprince, J., Tonon, M. C., Vaudry, H. & Costentin, J. The octadecaneuropeptide ODN induces anxiety in rodents: possible involvement of a shorter biologically active fragment. *Peptides* **19**, 841–848 (1998).
42. Cavaillon, J. M. & Haeflner-Cavaillon, N. The role of serum in interleukin 1 production by human monocytes activated by endotoxins and their polysaccharide moieties. *Immunol. Lett.* **10**, 35–41 (1985).
43. de Mateos-Verchere, J. G., Leprince, J., Tonon, M. C., Vaudry, H. & Costentin, J. The octadecaneuropeptide [diazepam-binding inhibitor (33-50)] exerts potent anorexigenic effects in rodents. *Eur. J. Pharmacol.* **414**, 225–231 (2001).
44. do Rego, J.-C. et al. Pharmacological characterization of the receptor mediating the anorexigenic action of the octadecaneuropeptide: evidence for an endozepinergic tone regulating food intake. *Neuropsychopharmacology* **32**, 1641–1648 (2007).
45. Matsuda, K. et al. Effect of the diazepam-binding inhibitor-derived peptide, octadecaneuropeptide, on food intake in goldfish. *Neuroscience* **150**, 425–432 (2007).