

Original Research

Cite this article: Marazziti D, Betti L, Baroni S, Palego L, Mucci F, Carpita B, Cremone IM, Santini F, Fabbrini L, Pelosini C, Marsili A, Massimetti E, Giannaccini G, and Dell'Osso L (2022). The complex interactions among serotonin, insulin, leptin, and glycolipid metabolic parameters in human obesity. *CNS Spectrums* 27(1), 99–108. <https://doi.org/10.1017/S1092852920001820>

Received: 07 June 2020
Accepted: 13 August 2020




Key words

Obesity; serotonin; serotonin transporter; insulin; leptin; glycolipid metabolism.

Author for correspondence:

*Donatella Marazziti, MD
Email: dmarazzi@psico.med.unipi.it

The complex interactions among serotonin, insulin, leptin, and glycolipid metabolic parameters in human obesity

Donatella Marazziti^{1,2,3*} , Laura Betti⁴, Stefano Baroni¹, Lionella Palego⁴, Federico Mucci^{1,5} , Barbara Carpita^{1,5} , Ivan Mirco Cremone¹, Ferruccio Santini⁶, Laura Fabbrini⁷, Caterina Pelosini⁶, Alessandro Marsili⁶, Enrico Massimetti⁸, Gino Giannaccini⁴ and Liliana Dell'Osso¹

¹Department of Clinical and Experimental Medicine, Section of Psychiatry, University of Pisa, Italy, ²Saint Camillus International University of Health and Medical Sciences, Rome, Italy, ³Brain Research Foundation Lucca, Italy, ⁴Department of Pharmacy, University of Pisa, Italy, ⁵Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Italy, ⁶Department of Clinical and Experimental Medicine, Endocrinology Unit, Obesity and Lipodystrophy Centre, University of Pisa, Pisa, Italy, ⁷Pharmacology and Pharmacogenetics, University Hospital Unit, University of Pisa, Pisa, Italy, and ⁸ASST, «Servizio Psichiatrico Diagnosi e Cura», Hospital of «Bergamo Ovest», SSD, Treviglio, Italy

Abstract

Objective. To provide evidence to the link between serotonin (5-HT), energy metabolism, and the human obese phenotype, the present study investigated the binding and function of the platelet 5-HT transporter (SERT), in relation to circulating insulin, leptin, and glycolipid metabolic parameters.

Methods. Seventy-four drug-free subjects were recruited on the basis of divergent body mass index (BMIs) (16.5–54.8 Kg/m²). All subjects were tested for their blood glycolipid profile together with platelet [³H]-paroxetine ([³H]-Par) binding and [³H]-5-HT reuptake measurements from April 1st to June 30th, 2019.

Results. The [³H]-Par B_{max} (fmol/mg proteins) was progressively reduced with increasing BMIs ($P < .001$), without changes in affinity. Moreover, B_{max} was negatively correlated with BMI, waist/hip circumferences (W/HC), triglycerides (TD), glucose, insulin, and leptin, while positively with high-density lipoprotein (HDL) cholesterol ($P < .01$). The reduction of 5-HT uptake rate (V_{max} , pmol/min/10⁹ platelets) among BMI groups was not statistically significant, but V_{max} negatively correlated with leptin and uptake affinity values ($P < .05$). Besides, [³H]-Par affinity values positively correlated with glycemia and TD, while [³H]-5-HT reuptake affinity with glycemia only ($P < .05$). Finally, these correlations were specific of obese subjects, while, from multiple linear-regression analysis conducted on all subjects, insulin ($P = .006$) resulting negatively related to B_{max} independently from BMI.

Conclusions. Present findings suggest the presence of a possible alteration of insulin/5-HT/leptin axis in obesity, differentially impinging the density, function, and/or affinity of the platelet SERT, as a result of complex appetite/reward-related interactions between the brain, gut, pancreatic islets, and adipose tissue. Furthermore, they support the foremost cooperation of peptides and 5-HT in maintaining energy homeostasis.

Introduction

Obesity is a major problem of industrialized countries with paramount individual, social, and economic implications.^{1,2} This is essentially due to the association of the obese phenotype with severe diseases at risk of premature death, such as severe depression, diabetes, metabolic syndrome, cardiovascular illnesses, and some types of cancers.^{3–7} The crucial question is why many people eat in excess of their metabolic needs, developing social maladjustment and overweight-related diseases. Among causes thought to engender unbalanced energy expenditure and adiposity gain, there are genetic vulnerabilities, sedentary lifestyle, endocrine illnesses, some pharmacological treatments, and, noticeably, mental factors, such as abnormal stress responses, anxiety, food addiction, and compulsive food intake.^{2,5,8,9}

Eating is a complex behavior, requiring the coordination of central and peripheral nervous systems, as well as of endocrine system, and of peptides, such as leptin, ghrelin, insulin, and neuropeptide Y.^{8,9} Leptin is secreted from adipocytes and is a mediator of long-term regulation of energy balance, by suppressing food intake and thereby inducing weight loss, while ghrelin is a fast-acting hormone, playing a role in meal initiation.^{8,9} Leptin inhibits the hypothalamic-pituitary-adrenal axis (HPA), the CRF mRNA expression, and glucocorticoid levels, as well as the rewarding properties of feeding, while ghrelin exerts opposite activities.^{8,9}

Several converging studies of the last 40 years suggest that serotonin (5-HT) may be implicated in the control of satiety and feeding behaviors with different receptors, specifically 5-HT_{1B}, 5-HT_{2C}, and 5-HT₆ at the level of the hypothalamic ventromedial and lateral nuclei, while interacting with both orexin and α -melanocortin-stimulating hormone (α -MSH), and in the nucleus of the solitary tract by integrating peripheral satiety inputs.¹⁰ Intriguingly, the 5-HT neurotransmission and receptor functions are influenced by glucocorticoid response in the central nervous system (CNS), affecting feeding behavior and the choice of macronutrient composition in diet that, in turn, is able to influence 5-HT synthesis.^{11–15} Furthermore, 5-HT improves the glucose homeostasis, lipid metabolism, bone density, and some conditions associated with metabolic syndrome, such as obesity,^{12,16} and it is also synthesized in pancreatic islets and co-secreted along with insulin, potentially acting as a local autocrine/paracrine signal.¹⁶ Again, 5-HT pathways are involved in the adaptation to cold exposure through the sympathetic activation of thermogenic adipose tissue, as adipocytes express 5-HT receptor subtypes¹⁷ and the 5-HT reuptake carrier (5-HT transporter, SERT).¹⁸ The adipocyte-derived 5-HT has been implicated in lipogenesis, with some activity also on leptin.^{16,19} Intriguingly, leptin has been shown to inhibit appetite by decreasing the 5-HT synthesis or release by the serotonergic neurons of the brainstem, targeting arcuate nucleus 5HT_{1A} and 5-HT_{2B} receptors.^{20,21}

Not surprisingly, 5-HT, leptin, ghrelin, and several other peptides have been suggested to play a role in the pathophysiology of different eating disorders.²²

One possibility to study 5-HT in humans *in vivo* is represented by blood cells, such as platelets or lymphocytes.^{23,24} Both platelets and lymphocytes possess the protein carrier for 5-HT uptake, SERT, identical to that found on presynaptic serotonergic neurons, as shown by pharmacological and cloning studies.²⁵ For this reason, they have been widely used in biological psychiatry as a reliable model of the same CNS structure.^{26–30} In addition, platelets respond to agonist-induced activation by rapid phosphorylation and represent a good model to study signal transduction events.³⁰ In this context, SERT, as other carriers belonging to the superfamily of monoamine transporters, undergoes a fine-tuned short-term and long-term regulation to adapt 5-HT levels in cells, tissues, and body fluids. Several trafficking proteins, proteases, phosphatase, and kinase activities may change SERT at the level of its gene expression, density, protein turn-over, and conformational states, modifying the affinity and functional kinetics of the carrier.^{31,32} Moreover, the SERT protein is a target of different psychotropic compounds, primarily, the selective 5-HT reuptake inhibitors (SSRIs), the most common antidepressants (ADs) widely used in eating disorders.

Given the increasing role of 5-HT in metabolism, the aim of the present study was to evaluate, by extending a previous survey,¹¹ both platelet SERT binding and reuptake parameters, together with circulating leptin, insulin, triglycerides (TG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL), high-density lipoprotein-cholesterol (HDL), glucose, systolic and diastolic pressure, body mass index (BMI, Kg/m²), circumference of waist (WC), and hips (HC), as well as their possible interactions, in a group of obese individuals, also including the large obese ones, as compared with nonobese subjects. Based on theoretical and empirical arguments, we propose the following three hypotheses: presence of platelet SERT alterations in obesity; differential leptin, insulin values, and metabolic parameters values in different BMI classes; and eventual interrelationships between the SERT, leptin, insulin, and metabolic parameters.

Materials and Methods

Chemicals and reagents

[³H]-paroxetine (specific activity: 15–21 Ci/mmol) and [³H]-5-HT (28.1–30 Ci/mmol) were purchased from Perkin-Elmer, Life Science, Milan, Italy. All other reagents used were of the best analytical grade. High-performance liquid chromatography-grade water was employed for all solutions and buffers.

Subjects

Seventy-four subjects were enrolled for the present study and were recruited among patients and their nonobese accompanying persons at the Obesity and Lipodystrophy Center of the Department of Clinical and Experimental Medicine, Endocrinology Unit, University of Pisa. All overweight and obese individuals were consulting the Endocrinology Center to lose weight. Medical exclusion criteria, appraised by F.S. and A.M., were active cancer, heart, liver or kidney diseases, and presence of hematological or neurological illnesses. Another exclusion criterion was the use of hypotensive drugs or compounds interacting with carbohydrate-lipid metabolism (insulin, oral hypoglycemic compounds, and statins). The psychiatric exclusion criteria were presence of family or personal history of any major psychiatric disorder or substance abuse. Therefore, all subjects underwent a detailed, 1-hour long psychiatric interview conducted by FM, BC, IMC and EM and supervised by senior psychiatrists (DM and LD), to clinically exclude both major and minor psychiatric disorders. The absence of any major psychopathological disorder was subsequently confirmed by the structured clinical interview for DSM-IV-Patient Version 2.0 (SCID-P).³³ All recruited subjects had or were currently suffering from no eating or anxiety disorders. Three obese and four nonobese subjects reported the occasional use of different sleeping pills (melatonin, benzodiazepines, or Z-drugs). All subjects neither were heavy cigarette smokers nor belonged to groups of high-risk HIV individuals.

Subjects taking psychotropic drugs acting on the SERT, other psychotropic agents, estrogenic drugs, or nonsteroidal anti-inflammatory agents were included in the study after a 3 months and 10 days withdrawal, respectively.

Height was measured while subjects were standing, using standardized techniques and equipment. Body weight was measured by a precision instrument and an electronic scale (± 0.1 Kg). The subjects were classified according to the five classes of the BMI (Kg/m²) as follows: BMI between 18.5 and 24.9: normal weight (n = 18); BMI between 25 and 29.9: overweight (preobesity, n = 12); BMI between 30 and 35: class I obesity (n = 9); BMI between 35 and 39.9: severe obesity or class II obesity (n = 10); and BMI > 40: class III obesity (n = 25). Given the small numbers of subjects of the class I and II, they were merged together for statistical analyses.

All subjects signed the informed consent after a full explanation of the study that had been approved by the Ethics Committee at Pisa University.

Blood collection

Venous blood samples (35 mL) were obtained after an overnight fasting between 8 and 9 a.m. during the months of April to June 2019, in order to avoid the possible interference of circadian or seasonal rhythms, respectively.³⁴

Measurement of metabolic parameters

After sample withdrawal, 5 mL of the collected venous blood were immediately sent to the Laboratory of Clinical Chemistry, University Hospital of Pisa, for the determination of glucose, TD, TC, LDL-cholesterol, HDL-cholesterol, insulin, and leptin, measured by means of automatized procedures. Leptin and insulin were measured by ELISA methods (Mediagnost, Germany).

Platelet sampling

The remaining 30 mL blood samples were used for platelet preparation, after mixing them with 1 mL of EDTA 0.1 M. Platelet-rich plasma was obtained by low-speed centrifugation ($150 \times g$, for 20 minutes, at 22°C). Platelets were counted automatically by means of a flux cytometer (Cell-dyn 3500 system, Abbott, Milano, Italy).

For [³H]-5-HT reuptake, platelets were used immediately. For [³H]-Par binding, platelets were precipitated by centrifugation at $10\,000 \times g$ for 10 minutes at 4°C, and the ensuing pellets were then stored at -80°C until the assay, which was performed within a week.

[³H]-5-HT reuptake

For [³H]-5-HT reuptake assay, intact platelets were maintained under physiological conditions, such as isotonic buffer, presence of NaCl and glucose, 37°C assay temperature. Precisely, the assay was performed according to the method of Arora and Meltzer,³⁵ with some modifications.³⁴ Aliquots of platelets (2×10^6 cells)⁶ were incubated with [³H]-5-HT at six crescent concentrations ranging from 20 nM to 1500 nM in a Krebs assay buffer (118 mM NaCl, 4.7 mM KCl, 1.07 mM MgSO₄ 7H₂O, 1.17 mM KH₂PO₄, 25 mM NaHCO₃, 11.6 mM glucose, pH 7.4) to a final volume of 0.5 mL. The incubation was carried out for 10 minutes at 37°C. Nonspecific reuptake was measured in the presence of 10 μM fluoxetine (a gift from Eli Lilly Co, Indianapolis, IN). Specific reuptake was determined as the difference between total and nonspecific reuptake. All points were performed in duplicate. The reaction was halted by the addition of 5 mL of ice-cold assay buffer, subsequently followed by rapid filtration through Whatman GF/C glass-fiber filters. Filters were incubated with 200 μL of 0.2 N NaOH to obtain the cell lysis, neutralized by adding 200 μL of 0.2 N-glacial acetic acid, and placed in plastic vials with 10 mL of scintillation liquid for radioactivity measurement, performed through a liquid phase scintillation β-counter (Packard 1600 TR) and taken as dpm.

[³H]-Par binding

At the time of assay, platelets were thawed and homogenized by means of an Ultra-turrax homogenizer in 10 mL of ice-cold buffer (5 mM Tris-HCl, 5 mM EDTA, pH 7.4, 4°C), containing a cocktail of protease inhibitors (160 μg/ml benzamidine, 200 μg/ml bacitracine, 20 μg/ml Soy-Trypsin Inhibitor), and centrifuged at $48\,000 \times g$ for 15 minutes at 4°C. The pellet was resuspended in 10 mL of ice-cold 50 mM Tris-HCl buffer, and washed twice by centrifugation at $48\,000 \times g$ for 15 minutes at 4°C. The final precipitate was suspended in the assay buffer (50 mM Tris, 120 mM NaCl, 5 mM KCl pH 7.4) and homogenized. Protein content was determined by the Bradford method (Bio-rad), using γ-globulins as the standard. The [³H]-Par binding was carried out according to the method of Marazziti and coauthors.²⁷ The incubation mixture consisted of 100 μL of platelet membranes (50-100 μg protein/tube), 50 μL of

[³H]-Par at six concentrations ranging from 0.01 to 1 nM and 1.85 mL of assay buffer. Specific binding was obtained as that remaining in the presence of 10 μM fluoxetine as a displacer. All samples were assayed in duplicate and incubated at 22°C for 1 hour. The incubation was halted while adding 5 mL of cold assay buffer. The content of the tubes was immediately filtered under vacuum through Whatman glass-fiber filters GF/C and washed three times with 5 mL of assay buffer. Filters were then placed in vials with 4 mL of scintillation cocktail, and radioactivity (dpm) was measured by means of a β-counter (Packard LS 1600).

Calculations and statistical analyses

All demographic, clinical, and laboratory data were presented as mean ± standard deviation (SD), variation range (min and max values). The maximal velocity (V_{max} , pmol/min/ 10^9 platelets) and the Michaelis-Menten constant (K_M , nM) of the [³H]-5-HT reuptake were calculated by transforming saturation data according to the Lineweaver-Burk plot method with the aid of the GraphPad Prism program (version 7.0, San Diego, CA). Equilibrium-saturation binding data, the maximum binding capacity (B_{max} , fmol/mg protein), and the dissociation constant (K_D , nM) of [³H]-Par binding were analyzed by means of iterative curve-fitting computer programs, EBDA-LIGAND (Kell for Windows, version 6.0) and GraphPad Prism.³⁶

For gender comparisons on biological parameters, the Student's *t*-test for independent samples was used.

The correlation between the [³H]-5-HT, [³H]-Par binding, and the other parameters, and between characteristics of the subjects and biological markers were explored by calculating the Pearson's correlation coefficient.

The possible differences between obese and nonobese subjects of different categories were assessed by the ANOVA Bonferroni's *post hoc* comparisons.

Multiple linear-regression analyses were also carried out to appraise predictive independent variables on SERT parameters, taken as the dependent variables.

All statistical analyses were carried out using the GraphPad Prism software and the Statistical Package for Social Sciences (SPSS), version 23.

A two-sided *P* value of .05 was considered the statistical threshold.

Results

As shown in Table 1, the recruited subjects were prevalently women, with a mean age of 38.2 years. According to recruitment criteria, comprising normal weight, overweight, and obese subjects, as well as large obese ones, the BMI ranged between 16.5 and 54.8 kg/m² (34.38 ± 10.61). The systolic pressure (mmHg, mean ± SD) was 126.8 ± 12.3 and the diastolic pressure was 79.8 ± 11.0 , being on average within the normal range, as the metabolic parameters and platelet SERT binding or reuptake velocity measures (B_{max} and K_D ; V_{max} and K_M). About 40% of obese subjects (obese I/II and III ones) had high fasting insulin (> 20 μIU/mL) and leptin (> 30 ng/mL) levels. The mean levels of glucose were 94 ± 18 mg/dL, but 40% of obese subjects had value >100 mg/dL, while those of total cholesterol were 200 ± 11 mg/dL, with only 17% subjects beyond 200 mg/dL. No difference was detected between men and women, except that for leptin levels being significantly higher in men than in women (44.5 ± 30.6 vs 25.5 ± 26.9 , $P = .013$).

Table 1. The Anthropometric, Blood Pressure, Blood Metabolic Variables, and the Platelet SERT Parameters, Measured in the Whole Group of Subjects.

	All Subjects, n = 74
BMI, Kg/m ²	34.4 ± 10.61 (16.5-54.8)
Age, years	38.2 ± 12.5 (15-63)
Waist circumference, mm ^a	101.7 ± 24.2 (59-160)
Hip circumference, mm ^a	113 ± 21.2 (81-155)
Systolic blood pressure (SBP), mmHg	126.8 ± 12.3 (90-160)
Diastolic blood pressure (DBP), mmHg	79.8 ± 11.03 (55-100)
Total cholesterol, mg/dL	200 ± 41.7 (113-300)
LDL, mg/dL	130 ± 36.9 (46-217)
HDL, mg/dL	57 ± 15.6 (26-98)
Triglycerides (TD), mg/dL	124.8 ± 83.1 (37-477)
Glucose, mg/dL ^a	93.6 ± 18.3 (70-169)
Insulin, μIU/mL	13.2 ± 9.97 (1.0-56.1)
Leptin, ng/mL	38.5 ± 30.64 (0.7-116)
[³ H]-Par B _{max} (fmol/mg proteins)	1085 ± 350 (336-1716)
[³ H]-Par K _D , nM	0.08 ± 0.06 (0.025-0.47)
[³ H]-5-HT V _{max} (pmol/ /min/10 ⁹ platelets)	104.2 ± 55.26 (27.8-320)
[³ H]-5-HT K _M , nM	128.3 ± 168.4 (21.4-1198)

Data are presented as the mean ± SD and ranges (min and max values). Abbreviations: BMI, body mass index; HDL, high-density lipoprotein-cholesterol; LDL, low-density lipoprotein-cholesterol; NW, normal weight; OB, obese; OW, over weight. ^aThese parameters were appraised in n=41 subjects, NW: n=9; OW: n=9; OB-I/II: n=13; OB-III: n=10.

Table 2 reports the mean ± SD of all investigated variables, in their different BMI groups, together with corresponding results of ANOVA inferential tests. Prior to ANOVA and correlation tests, the [³H]-Par K_D and [³H]-5-HT K_M values, parameters linked to the carrier conformational, and ligand affinity state were $-\log [M]$ transformed (trK_D and trK_M), because they were not normally distributed.

The *post hoc* comparisons between nonobese and obese subjects showed that the [³H]-Par B_{max} values were progressively lowering from nonobese to class III obesity (F: 8.15. $P < .001$), while insulin and leptin were increasing (F: 14.00. $P < .001$), as they were TD, glucose, blood pressure values, and WC or HC measures. While HDL was progressively and significantly reducing with higher BMIs, there was no relevant increase in TC and LDL levels with the degree of obesity.

The Pearson's correlation analyses, carried out in the whole sample, revealed the presence of different relationships among biological parameters. Figure 1(A-C) shows that the platelet SERT density, or [³H]-Par B_{max}, was negatively correlated with BMI, and with WC and HC, anthropometric parameters linked to obesity. Figure 2(A-E) depicts the significant correlations between B_{max} and most circulating fasting metabolic indexes: negative with insulin, leptin, glucose, and TD, while positives with HDL. Concerning the SERT functionality, significant and negative correlations were found between the [³H]-5-HT reuptake V_{max}, leptin, and the trK_M itself (Figure 3A,B). Figure 4A-F displays that trK_D was significantly and positively correlated with glucose and TD, and trK_M, with glucose, WC, HC, and close to significance with leptin. When the subjects were distinguished in normal weighted and obese, no

significant correlations were present in the first group, while they remained in obese subjects.

Multiple linear-regression analysis, carried out considering SERT binding and function parameters as dependent variables, reported that increased insulinemia was predictive of reduced SERT B_{max} values ($P < .006$), independently from body weight and obesity degrees (Table 3).

Discussion

Obesity is a burden for the world public health due to its association with the risk of developing severe metabolic and cardiovascular illnesses. This motivated intense investigation on those neuroendocrine and biochemical substrates that underlie body fat mass accrue as possible targets for successful therapeutic interventions. 5-HT, a monoamine acting as a neurotransmitter in the brain and a pleiotropic signal in peripheral tissues, has been linked to eating behavior and obesity in animal models and humans. It is considered among satiety factors, being able to reduce food intake and meal size, while its downregulation bringing about the opposite effect.³⁷⁻³⁹ The functioning of the 5-HT network is tightly regulated by the balance between its biosynthesis rate from the essential amino acid tryptophan, its release, and catabolism,⁴⁰ and by the degree of occupation/sensitivity of the different 5-HT receptors and the activity of intra-cellular 5-HT reuptake via the protein carrier SERT.^{41,42} Moreover, peripheral 5-HT has been involved in the modulation of energy metabolism, stimulating the search for its networks linked to adipose tissue accumulation.^{16,43}

In the present study, we explored the relationships between the platelet SERT, a peripheral serotonergic marker, and TD, total, HDL and LDL cholesterol, insulin, glucose, leptin, blood pressure, BMI, WC and HC. The mean values of the platelet SERT parameters found in the overall subjects, included in this study, were within the range of healthy control individuals, as compared with those patients with different psychiatric conditions of our extensive database.^{23,27-29,33,44} This was expected, because we excluded all subjects with family or personal history of any major psychiatric disorder. Even the mean values of most glycolipid metabolic parameters in most subjects were within the normal range. These results highlighted that the majority of the subjects, in spite of the evidence of being more or less obese, did not show any severe overt metabolic disturbances. This might be due to the fact that all were Italian and, although coming from different regions, they share common food habits and recipes. Taken together, these findings again support the paramount benefits of Mediterranean diet.^{45,46}

However, the *post hoc* comparisons of nonobese vs obese subjects of different BMI classes revealed significant differences. The density of the SERT (B_{max}) was significantly higher in the control subjects than in all BMI classes that were different one from another, confirming previous investigations conducted in platelets¹¹ or in brain cortical and subcortical regions.⁴⁷ Furthermore, to our knowledge, this is the first study investigating SERT functionality and 5-HT reuptake kinetics in obesity: reduced SERT binding was paralleled by a similar trend in V_{max} values, although these findings would indicate progressive and significant changes of the 5-HT system in parallel with increasing weight, until the point that SERT expression (and, perhaps, functionality) values are similar to those found in patients with different psychiatric disorders.^{27,28,33,44} The reduced SERT number, therefore, might constitute, not only a marker of vulnerability toward the onset of

Table 2. The Investigated Anthropometric, Blood Pressure, Blood Metabolic Variables, and the Platelet SERT Parameters, Divided with Respect to BMI.

	Normal Weight	Overweight	OB Grade I/II	OB Grade III
	(NW, n=19)	(OW, n=11)	(OB-I/II, n=20)	(OB-III, n=24)
BMI, Kg/m ²	21.2 ± 2.3 (16.5-25)	27 ± 1.2 ^{***,****} (25.4-28.8)	36 ± 2.8 ^{***,****} (31.2-40)	46.7 ± 4.1 ^{***,****} (41-54.8)
Age, years	35.3 ± 12.9 (20-61)	37.3 ± 14 (21-57)	37.5 ± 13.3 (15-63)	41.4 ± 10.8 (23-59)
Waist circumference, mm ^a	71 ± 8.5 (59-85)	88.7 ± 9.7 ^{***} (78-108)	110 ± 6.8 ^{***} (99-122)	130 ± 17.34 ^{***} (102-160)
Hip circumference, mm ^a	89.3 ± 5.7 (81-101)	98 ± 7.1 (88-105)	121 ± 10.8 ^{***} (105-143)	137 ± 12.3 ^{***} (116-155)
Systolic blood pressure (SBP), mmHg	117 ± 7.5 (105-135)	124 ± 9.3 (110-140)	131 ± 11 ^{**} (110-150)	132 ± 13.6 ^{***} (90-160)
Diastolic blood pressure (DBP), mmHg	73.7 ± 8.4 (58-90)	76.6 ± 8.4 (65-90)	81.9 ± 11.7 (55-100)	84.5 ± 11.2 ^{**} (60-100)
Total cholesterol, mg/dL	200 ± 53.6 (113-298)	190 ± 28.7 (157-248)	204 ± 41 (127-297)	204 ± 38 (139-300)
LDL, mg/dL	122 ± 46.6 (60-217)	128 ± 25 (96-182)	131 ± 35 (46-192)	137 ± 35.4 (84-207)
HDL, mg/dL	70.3 ± 14.64 (49-98)	51.2 ± 11.6 ^{**} (26-66)	55.7 ± 17.4 ^{**} (37-97)	50.4 ± 9.3 ^{***} (30-67)
Triglycerides (TD), mg/dL	66.2 ± 20.1 (37-112)	105 ± 38.4 (63-185)	160 ± 95.2 ^{**} (68-379)	151 ± 92.8 ^{**} (64-477)
Glucose, mg/dL ^a	84 ± 8.9 (70-96)	86 ± 8.8 (74-101)	94.5 ± 12.9 (72-115)	108 ± 27.4 [*] (76-169)
Insulin, µIU/mL	5.0 ± 2.4 (1.0-11.2)	7.7 ± 4 (5.0-16.7)	18.4 ± 7.5 ^{***} (6.8-31.7)	17.9 ± 11.8 ^{***} (5.0-56.1)
Leptin, ng/mL	12.1 ± 14.9 (1.0-65)	16.3 ± 13.9 (0.7-39.4)	51.5 ± 29.7 ^{***} (15-116)	58.9 ± 24.5 ^{***} (2.9-99)
[³ H]-Par B _{max} (fmol/mg proteins)	1277 ± 282 (767-1716)	1317 ± 153 (1158-1685)	1032 ± 421 (344-1700)	870 ± 251 (336-1278) ^{***,***}
[³ H]-Par K _D , nM	0.08 ± 0.04 (0.03-0.18)	0.07 ± 0.04 (0.03-0.16)	0.08 ± 0.09 (0.038-0.47)	0.07 ± 0.03 (0.025-0.094)
[³ H]-5-HT V _{max} (pmol/min/10 ⁹ platelets)	122 ± 43.2 (58.4-212.5)	82.5 ± 36 (47-137.2)	112 ± 69.6 (27.8-320)	93.6 ± 54.9 (28.4-254.9)
[³ H]-5-HT K _M , nM	139 ± 124 (31.2-522)	77 ± 43.4 (28.7-169)	102 ± 77.7 (23-303)	165 ± 262 (21.4-1198)

Data are presented as the mean ± SD and ranges (min and max values).

ANOVA, significant Bonferroni *post hoc* tests: NW vs OW or OB groups.

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein-cholesterol; LDL, low-density lipoprotein-cholesterol; NW, normal weight; OB, obese; OW, over weight.

^aThese parameters were appraised in n=41 subjects, NW: n=9; OW: n=9; OB-I/II: n=13; OB-III: n=10.

^{***}P < .0001.

^{**}P < .0001.

^{*}P < .001.

[~]P < .05; OW vs OB groups.

psychopathology, but an additional cue of severe obesity and/or of those metabolic disorders quite present in different psychiatric conditions, while perhaps suggesting a common diathesis.⁴⁸ This is hence supported by the findings of significantly lower and higher levels of, respectively, HDL and TD, leptin, insulin, or glucose in obese vs control subjects, with a constant progression along the different classes of obesity. According to us, the changes in fasting insulin and leptin values from overweight to class III subjects are really impressive.

The differences between nonobese and obese subjects are confirmed by the presence of several and significant correlations among the different parameters. The most numerous were negative and between the B_{max} and BMI, WC, HC, TD, glucose, insulin, and leptin. On the contrary, B_{max} resulted to be positively related to HDL cholesterol, a marker of lipid metabolism endowed with athero- and cardioprotective actions.⁴⁹ As the B_{max} is a measurement of the density of the SERT proteins, these findings reveal that the higher these values, the lower BMI, TD, glucose, insulin, leptin, WC and HC, and vice versa. On the contrary, the higher the B_{max} values, the higher the HDL cholesterol. Intriguingly, the V_{max}, which is the velocity of the reuptake of 5-HT, was negatively correlated with leptin. Both the trK_D and trK_M, that measure the affinity of the ligand, that is to say, 5-HT to its site, were positively related to TD and glucose the first, and to glucose the second. Moreover, these significant correlations were specific for obese

subjects only, suggesting that adiposity is able to modulate platelet SERT.

These results generate other reflections. First, a normal density and functioning of the SERT seem to be related to “healthy” values of different parameters, specifically of lipid and glucose metabolism, while a decreased SERT is a cue or a consequence of, or provoke metabolic alterations, or, probably, as mentioned, share the same pathophysiology. In this regard, it should be mentioned that increased glycemia values and decreased HDL levels, as reported here in subjects with higher BMIs and lower B_{max}, have been linked to the presence of inflammatory patterns and increased oxidative stress,⁵⁰⁻⁵² similarly, in part, to what reported in mood disorders.⁵³⁻⁵⁵ Of note, obesity and psychiatric disorders may share impairment in energy metabolism.⁵⁶

Adipokines are thought to be among the mediators of such common features.^{57,58} Leptin has been considered as an anti-obesity agent, acting as a signal of an adiposity negative feedback⁵⁹: low leptin prevent from starvation, while its release by adipocytes is related to plenty of food intake.⁶⁰ Although the links between leptin and 5-HT are unclear,⁶¹ different data support the close relationships between them as the peptide inhibits appetite by suppressing the biosynthesis and release of 5-HT from 5-HT neurons in the brainstem.^{20,21} These findings seem in contrast to the decreased density of the SERT in obese individuals, as we observed a negative relationship between the two parameters. Anyway, the literature is

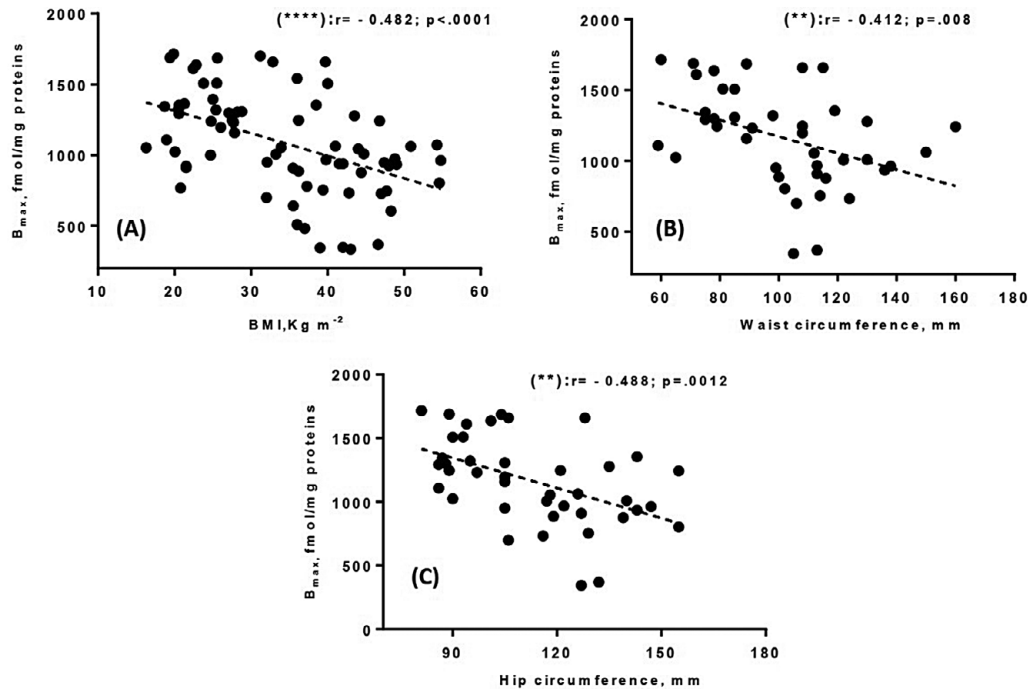


Figure 1. Significant correlations of platelet [^3H]-Par B_{max} with anthropometric indices: (A) body mass index (BMI); (B) waist circumference (WC); (C) hip circumference (HC). The Pearson's coefficient of correlation r and corresponding P values are depicted; the dotted lines represent the best fit from linear-regression analysis. ****Strong and **high-to-moderate statistical significance of the Pearson's correlation.

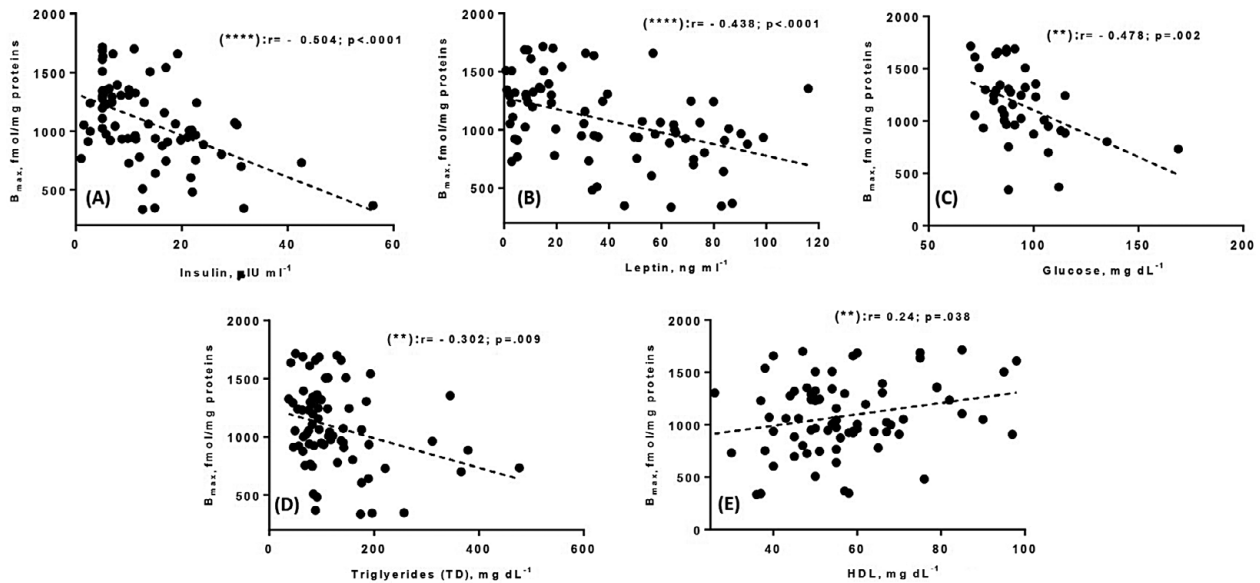


Figure 2. Significant correlations of platelet [^3H]-Par B_{max} with glycolipid metabolic parameters: (A) insulin; (B) leptin; (C) glucose; (D) triglycerides (TD); (E) the high-density lipoprotein-cholesterol (HDL). The Pearson's coefficient of correlation r and corresponding P values are depicted; the dotted lines represent the best fit from linear-regression analysis. ****Strong, **high-to-moderate, and * moderate statistical significance of the Pearson's correlation.

consistent in reporting high leptin levels in obesity, so that a condition of leptin resistance is considered as one of the factors provoking obesity.^{62–64} Obese subjects, in spite of the high levels of leptin, indeed, would continue to eat. Interestingly, although data are conflicting, increased levels of leptin have been associated also with 20% prevalence of depression in obese subjects.⁶⁵ In light of these findings, it would be interesting to know whether leptin may trigger depression only in those obese subjects with a decreased density of the SERT and reduced 5-HT uptake.

We also observed a decreasing expression of platelet SERT related to increasing levels of insulin along the different degrees of obesity. Interestingly, high level of glycemia, linked to insulin resistance, was positively correlated with SERT affinity (trKD and trKM), implying a higher affinity for 5-HT under this condition. Insulin is the key hormone in ensuring energy reserves, through its action of strengthening anabolic pathways after food intake as the synthesis of glycogen and proteins, the activation of lipogenesis and fat storage in adipose tissue. In an attempt to explain the present

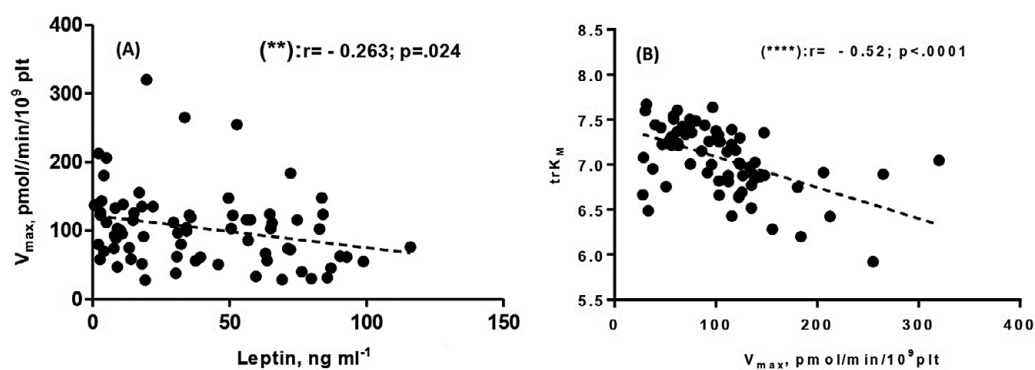


Figure 3. Significant correlations of platelet [³H]-5-HT reuptake V_{max} with leptin and trK_M ($-\log[M]K_M$). The Pearson's coefficient of correlation r and corresponding P values are depicted; the dotted lines represent the best fit from linear-regression analysis. ****Strong and **high-to-moderate statistical significance of the Pearson's correlation.

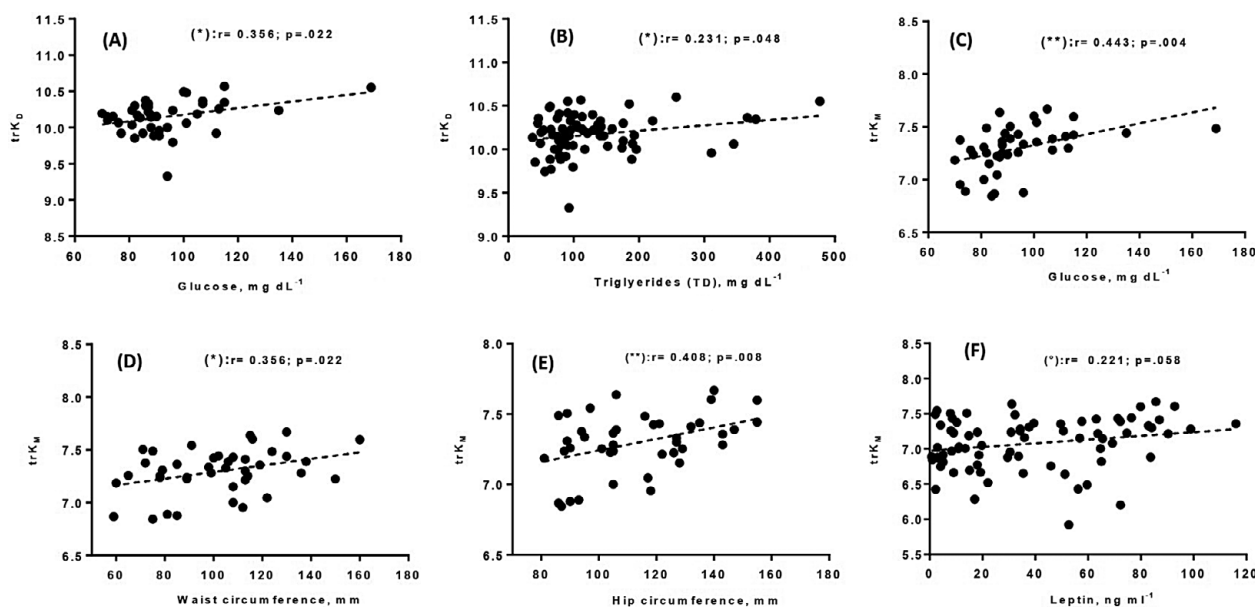


Figure 4. Correlations of the platelet [³H]-Par and [³H]-5-HT reuptake affinity measures with glycolipid metabolic parameters: trK_D ($-\log[M]K_D$) vs (A) glucose; (B) triglycerides (TD); trK_M ($-\log[M]K_M$) vs (C) waist circumference (WC); (D) hip circumference (HC); (E) leptin. The Pearson's coefficient of correlation r and corresponding P values are depicted; the dotted lines represent the best fit from linear-regression analysis. **High-to-moderate, *moderate, and *trend for statistical significance of the Pearson's correlation.

Table 3. Significant Multiple Regression Analysis with [³H]-Par B_{max} as the Dependent Variable.

	Nonstandardized Coefficients		Standard Coefficients		
	<i>B</i>	Standard Error	Beta	<i>t</i> -value	<i>P</i> value
Blood insulin	-12.84	4.28	-.442	-2.994	.006

results, it should be considered that insulin, stimulated by meal consumption, promotes the synthesis and release of 5-HT (brain) and leptin (adipocytes). “Gene × environment” interactions and/or different triggers and disruptors are supposed to impact this endocrine pathway at various degrees, also at the level of the 5-HT system in vulnerable individuals: among these variables, there are genetic polymorphisms, stressors, and the stress response, lifestyles inducing carbohydrate craving, and reward circuits, as well as inflammation patterns.^{9,13,14,66,67} Ensuing 5-HT changes might entail a different metabolism, a reduced sensitivity of 5-HT_{2C} and 5-HT_{1B} receptors,³⁷⁻³⁹ in respect to the 5-HT_{1A} and 5-HT_{2B} receptors in the hypothalamus,^{20,21} and/or to the 5-HT₆ ones in the hypothalamus and nucleus accumbens,⁶⁸ in favor of an increased motivation to eat. Increased food intake would stimulate peripheral

5-HT generated from the gut and pancreatic cells to induce more insulin secretion, body fat increase, further release of leptin, and, ultimately, insulin and leptin resistance, reinforcing hunger circuits.^{16,69} The normal cooperation between insulin and 5-HT is lost, and insulin, instead of being hampered, continues to be produced by pancreatic islets.⁷⁰ This is a condition of insulin resistance widely reported in obesity, diabetes, and depression.⁷¹ Therefore, the reduced platelet SERT density with increased BMI would reflect the defective and adipogenic activity of this brain-periphery endocrine loop, or the insulin-5-HT-leptin axis.

Another important result of this study is that insulin was found to significantly predict the reduction of platelet SERT number, independently from BMI, confirming the tight physiological relationship between insulin, 5-HT, and energy metabolism by brain-

periphery pathways.^{40,70} Insulin and 5-HT cooperate to regulate energy homeostasis and eating behavior, indicating that alterations in their reciprocal interactions are linked to a variety of metabolic disorders, as diabetes and obesity, further providing a rationale to their comorbidity with depression.^{65,69,71,72}

In summary, alterations of the insulin-5-HT-leptin axis, accompanied by conditions of leptin and insulin resistance, would distinctively regulate the density, function, and/or affinity of platelet SERT by complex adaptive and appetite/reward-related cross talks involving the brain, the gut, pancreatic islets, and adipose tissue in the obese phenotype. The impairment of the insulin-5-HT cooperation seems to be a main effector of the observed platelet SERT number downregulation. On the other hand, although insulin reduces the platelet SERT number in a BMI-independent manner, this does not exclude that SERT expression, under a condition of insulin resistance, would be diminished by other blood obesity factors in grade II or III obese subjects. Further investigation is required to ascertain which signaling pathways related to obesity have the greatest impact on the regulation of platelet SERT.

Limitations

The present study shows some limitations that should be underlined. First, the relatively small number of subjects per category included in the sample, so that further studies are warranted to confirm or reject the present findings. Second, two-thirds of the obese subjects were women and one-third were men: in any case, all were outpatients and within a limited age range. Third, given the small numbers of subjects of the class I and II, they were merged together for statistical analyses: this might have prevented us to detect differences between these two classes of obesity. Fourth, platelet SERT is a peripheral measure and, even though it is widely used to investigate central serotonergic function, the surrounding environment could be quite different.²⁹

Conclusions

Taken together, our findings further support the role of the 5-HT system, specifically of the SERT in the regulation of feeding and metabolism.^{73,74} Consequently, although the complex interactions between 5-HT and other satiety and appetite factors are not yet elucidated, nevertheless, the serotonergic system remains among the major targets for future anti-obesity drugs.

Disclosures. Donatella Marazziti, Laura Betti, Stefano Baroni, Lionella Palego, Federico Mucci, Barbara Carpita, Ivan Mirco Cremone, Ferruccio Santini, Laura Fabbri, Caterina Pelosini, Alessandro Marsili, Enrico Massimetti, Gino Giannaccini, and Liliana Dell'Osso do not have any disclosures, and the coauthors do not have any affiliation with or financial interest in any organization that might pose a conflict of interest.

Authorship Contributions. D.M. and L.B.: conceived and designed the investigation; analyzed and interpreted the data; wrote the paper. G.G. and L.D.: conceived and designed the investigation; supervised the study and revised the final manuscript. F.S. and A.M.: conceived and designed the investigation; recruited subjects based on the inclusion/exclusion criteria; analyzed and interpreted the data. D.M., and L.D.: conducted the psychiatric interview. S.B., L.P., L.F., and C.P.: Designed the experiments and prepared the database; analyzed and interpreted the data; wrote the paper. S.B., L.F., and C.P.: supervised blood samplings and separated platelets; carried out experiments. F.M., B.C., I.M.C. and E.M.: participated to subjects' selection; analyzed and interpreted the data.

References

- Williams EP, Mesidor M, Winters K, Dubbert PM, Wyatt SB. Overweight and obesity: prevalence, consequences, and causes of a growing public health problem. *Curr Obes Rep.* 2015;4(3):363–370. doi:10.1007/s13679-015-0169-4.
- Apovian CM. Obesity: definition, comorbidities, causes, and burden. *Am J Manag Care.* 2016;22(suppl 7):s176–s185
- GBD 2016 Risk Factors Collaborators. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016 [published correction appears in *Lancet.* 2017;390(10104):1736] [published correction appears in *Lancet.* 2017;390(10106):e38]. *Lancet.* 2017;390(10100):1345–1422. doi:10.1016/S0140-6736(17)32366-8.
- Hales CM, Carroll MD, Fryar CD, Ogden CL. Prevalence of obesity among adults and youth: United States 2015–2016. *NCHS Data Brief.* 2016;2017(288):1–8.
- World Health Organisation. Obesity and overweight. <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight>. Accessed February 29, 2020.
- World Health Organisation. Global action plan for the prevention and control of non-communicable diseases 2013–2020. https://www.who.int/nmh/events/ncd_action_plan/en/. Accessed February 29, 2020.
- Hruby A, Hu FB. The epidemiology of obesity: a big picture. *Pharmacoeconomics.* 2015;33(7):673–689. doi:10.1007/s40273-014-0243-x.
- Volkow ND, Wang GJ, Baler RD. Reward, dopamine and the control of food intake: implications for obesity. *Trends Cogn Sci.* 2011;15(1):37–46. doi:10.1016/j.tics.2010.11.001.
- Field BC. Neuroendocrinology of obesity. *Br Med Bull.* 2014;109:73–82. doi:10.1093/bmb/ldu001.
- Voigt JP, Fink H. Serotonin controlling feeding and satiety. *Behav Brain Res.* 2015;277:14–31. doi:10.1016/j.bbr.2014.08.065.
- Giannaccini G, Betti L, Palego L, *et al.* The expression of platelet serotonin transporter (SERT) in human obesity. *BMC Neurosci.* 2013;14:128. doi:10.1186/1471-2202-14-128.
- Martin AM, Young RL, Leong L, *et al.* The diverse metabolic roles of peripheral serotonin. *Endocrinology.* 2017;158(5):1049–1063. doi:10.1210/en.2016-1839.
- Wurtman RJ. Non-nutritional uses of nutrients. *Eur J Pharmacol.* 2011;668(suppl 1):S10–15. doi:10.1016/j.ejphar.2011.07.005.
- Fernstrom JD. Large neutral amino acids: dietary effects on brain neurochemistry and function. *Amino Acids.* 2013;45(3):419–430. doi:10.1007/s00726-012-1330-y.
- Peuhkuri K, Sihvola N, Korpela R. Diet promotes sleep duration and quality. *Nutr Res.* 2012;32(5):309–319. doi:10.1016/j.nutres.2012.03.009.
- Wyler SC, Lord CC, Lee S, Elmquist JK, Liu C. Serotonergic control of metabolic homeostasis. *Front Cell Neurosci.* 2017;11:277. doi:10.3389/fncel.2017.00277.
- Kinoshita M, Ono K, Horie T, *et al.* Regulation of adipocyte differentiation by activation of serotonin (5-HT) receptors 5-HT2AR and 5-HT2CR and involvement of microRNA-448-mediated repression of KLF5. *Mol Endocrinol.* 2010;24(10):1978–1987. doi:10.1210/me.2010-0054.
- Stunes AK, Reseland JE, Hauso O, *et al.* Adipocytes express a functional system for serotonin synthesis, reuptake and receptor activation. *Diabetes Obes Metab.* 2011;13(6):551–558. doi:10.1111/j.1463-1326.2011.01378.x.
- Marazziti D, Rutigliano G, Baroni S, Landi P, Dell'Osso L. Metabolic syndrome and major depression. *CNS Spectr.* 2014;19(4):293–304. doi:10.1017/S1092852913000667.
- Oury F, Karsenty G. Towards a serotonin-dependent leptin roadmap in the brain. *Trends Endocrinol Metab.* 2011;22(9):382–387. doi:10.1016/j.tem.2011.04.006.
- Yadav VK, Oury F, Tanaka KF, *et al.* Leptin-dependent serotonin control of appetite: temporal specificity, transcriptional regulation, and therapeutic implications [published correction appears in *J Exp Med.* 2011;208(2):413. Tanaka, Kenji [corrected to Tanaka, Kenji F]]. *J Exp Med.* 2011;208(1):41–52. doi:10.1084/jem.20101940.

22. Tortorella A, Brambilla F, Fabrazzo M, *et al.* Central and peripheral peptides regulating eating behaviour and energy homeostasis in anorexia nervosa and bulimia nervosa: a literature review. *Eur Eat Disord Rev.* 2014; **22**(5):307–320. doi:10.1002/erv.2303).
23. Stahl SM. The human platelet. A diagnostic and research tool for the study of biogenic amines in psychiatric and neurologic disorders. *Arch Gen Psychiatry.* 1977; **34**(5):509–516. doi:10.1001/archpsyc.1977.01770170019001.
24. Marazziti D, Rossi A, Giannaccini G, Baroni S, Lucacchini A, Cassano GB. Presence and characterization of the serotonin transporter in human resting lymphocytes. *Neuropsychopharmacology.* 1998; **19**(2):154–159. doi: 10.1016/S0893-133X(97)00204-2.
25. Lesch KP, Wolozin BL, Murphy DL, Reiderer P. Primary structure of the human platelet serotonin uptake site: identity with the brain serotonin transporter. *J Neurochem.* 1993; **60**(6):2319–2322. doi:10.1111/j.1471-4159.1993.tb03522.x.
26. Langer SZ, Galzin AM, Poirier MF, Loo H, Sechter D, Zarifian E. Association of [³H]-imipramine and [³H]-paroxetine binding with the 5HT transporter in brain and platelets: relevance to studies in depression. *J Recept Res.* 1987; **7**(1–4):499–521. doi: 10.3109/10799898709054999.
27. Marazziti D, Rossi A, Gemignani A, *et al.* Decreased platelet ³H-paroxetine binding in obsessive-compulsive patients. *Neuropsychobiology.* 1996; **34**(4):184–187. doi:10.1159/000119308.
28. Marazziti D, Dell’Osso L, Rossi A, *et al.* Decreased platelet [³H]paroxetine binding sites in suicide attempters. *Psychiatry Res.* 2001; **103**(2–3):125–131. doi: 10.1159/000129665.
29. Marazziti D, Landi P, Baroni S, *et al.* The role of platelet/lymphocyte serotonin transporter in depression and beyond. *Curr Drug Targets.* 2013; **14**(5):522–530. doi:10.2174/1389450111314050003.
30. Aharonovitz O, Granot Y. Stimulation of mitogen-activated protein kinase and Na⁺/H⁺ exchanger in human platelets. Differential effect of phorbol ester and vasopressin. *J Biol Chem.* 1996; **271**(28):16494–16499. doi:10.1074/jbc.271.28.16494.
31. Carneiro AM, Blakely RD. Serotonin-, protein kinase C-, and Hic-5-associated redistribution of the platelet serotonin transporter. *J Biol Chem.* 2006; **281**(34):24769–24780. doi:10.1074/jbc.M603877200.
32. Giannaccini G, Betti L, Palego L, *et al.* Human serotonin transporter expression during megakaryocytic differentiation of MEG-01 cells. *Neurochem Res.* 2010; **35**(4):628–635. doi:10.1007/s11064-009-0112-8.
33. First MB, Spitzer RL, Gibbon M, Williams JBW. *Structured Clinical Interview for DSM-IV Axis I Disorders—Clinician Version (SCID-CV)*. Washington, DC: American Psychiatric Press; 1997.
34. Marazziti D, Falcone MF, Castrogiovanni P, Cassano GB. Seasonal serotonin uptake changes in healthy subjects. *Mol Chem Neuropathol.* 1990; **13**(1–2):145–154. doi:10.1007/BF03159915.
35. Arora RC, Meltzer HY. Serotonin uptake by blood platelets of schizophrenic patients. *Psychiatry Res.* 1982; **6**(3):327–333. doi: 10.1016/0165-1781(82)90022-1.
36. McPherson GA. Analysis of radioligand binding experiments. A collection of computer programs for the IBM PC. *J Pharmacol Methods.* 1985; **14**(3):213–228. doi:10.1016/0160-5402(85)90034-8.
37. Curzon G. Serotonin and appetite. *Ann N Y Acad Sci.* 1990; **600**:521–531. doi:10.1111/j.1749-6632.1990.tb16907.x.
38. Berglund ED, Liu C, Sohn JW, *et al.* Serotonin 2C receptors in pro-opiomelanocortin neurons regulate energy and glucose homeostasis [published correction appears in *J Clin Invest.* 2014; **124**(4):1868. Dosage error in article text]. *J Clin Invest.* 2013; **123**(12):5061–5070. doi:10.1172/JCI70338.
39. Garfield AS, Heisler LK. Pharmacological targeting of the serotonergic system for the treatment of obesity. *J Physiol.* 2009; **587**(1):49–60. doi: 10.1113/jphysiol.2008.164152.
40. Höglund E, Øverli Ø, Winberg S. Tryptophan metabolic pathways and brain serotonergic activity: a comparative review. *Front Endocrinol (Lausanne).* 2019; **10**:158. doi:10.3389/fendo.2019.00158.
41. Daws LC, Gould GG. Ontogeny and regulation of the serotonin transporter: providing insights into human disorders. *Pharmacol Ther.* 2011; **131**(1):61–79. doi:10.1016/j.pharmthera.2011.03.013.
42. Brindley RL, Bauer MB, Blakely RD, Currie KPM. An interplay between the serotonin transporter (SERT) and 5-HT receptors controls stimulus-secretion coupling in sympathoadrenal chromaffin cells. *Neuropharmacology.* 2016; **110**(pt A):438–448. doi:10.1016/j.neuropharm.2016.08.015.
43. El-Merahbi R, Löffler M, Mayer A, Sumara G. The roles of peripheral serotonin in metabolic homeostasis. *FEBS Lett.* 2015; **589**(15):1728–1734. doi:10.1016/j.febslet.2015.05.054.
44. Marazziti D, Rossi A, Palego L, *et al.* Effect of aging and sex on the [³H]-paroxetine binding to human platelets. *J Affect Disord.* 1998; **50**(1):11–15. doi:10.1016/s0165-0327(97)00074-8.
45. Fond G, Young AH, Godin O, *et al.* Improving diet for psychiatric patients: High potential benefits and evidence for safety. *J Affect Disord.* 2020; **265**:567–569. doi:10.1016/j.jad.2019.11.092.
46. Shafiei F, Salari-Moghaddam A, Larijani B, Esmailzadeh A. Adherence to the Mediterranean diet and risk of depression: a systematic review and updated meta-analysis of observational studies [published correction appears in *Nutr Rev.* 2019; **77**(6):454]. *Nutr Rev.* 2019; **77**(4):230–239. doi: 10.1093/nutrit/nuy070.
47. Erritzoe D, Frokjaer VG, Haahr MT, *et al.* Cerebral serotonin transporter binding is inversely related to body mass index. *Neuroimage.* 2010; **52**(1):284–289. doi:10.1016/j.neuroimage.2010.03.086.
48. Rajan TM, Menon V. Psychiatric disorders and obesity: a review of association studies. *J Postgrad Med.* 2017; **63**(3):182–190. doi:10.4103/jpgm.-JPGM_712_16.
49. Navab M, Reddy ST, Van Lenten BJ, Fogelman AM. HDL and cardiovascular disease: atherogenic and atheroprotective mechanisms. *Nat Rev Cardiol.* 2011; **8**(4):222–232. doi:10.1038/nrcardio.2010.222.
50. Rytter E, Vessby B, Asgård R, *et al.* Glycaemic status in relation to oxidative stress and inflammation in well-controlled type 2 diabetes subjects. *Br J Nutr.* 2009; **101**(10):1423–1426. doi:10.1017/s0007114508076204.
51. Norata GD, Pirillo A, Ammirati E, Catapano AL. Emerging role of high-density lipoproteins as a player in the immune system. *Atherosclerosis.* 2012; **220**(1):11–21. doi:10.1016/j.atherosclerosis.2011.06.045.
52. Holven KB, Retterstol K, Ueland T, *et al.* Subjects with low plasma HDL cholesterol levels are characterized by an inflammatory and oxidative phenotype. *PLoS One.* 2013; **8**(11):e78241. doi:10.1371/journal.pone.0078241.
53. Maes M, Yirmiya R, Norberg J, *et al.* The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. *Metab Brain Dis.* 2009; **24**(1):27–53. doi: 10.1007/s11011-008-9118-1.
54. Zunsain PA, Heppul N, Pariante CM. Inflammation and depression. *Curr Top Behav Neurosci.* 2013; **14**:135–151. doi:10.1007/7854_2012_211.
55. Felger JC. Imaging the role of inflammation in mood and anxiety-related disorders. *Curr Neuropharmacol.* 2018; **16**(5):533–558. doi:10.2174/1570159X15666171123201142.
56. Zuccoli GS, Saia-Cereda VM, Nascimento JM, Martins-de-Souza D. The energy metabolism dysfunction in psychiatric disorders postmortem brains: focus on proteomic evidence. *Front Neurosci.* 2017; **11**:493. doi: 10.3389/fnins.2017.00493.
57. Iikuni N, Lam QL, Lu L, Matarese G, La Cava A. Leptin and inflammation. *Curr Immunol Rev.* 2008; **4**(2):70–79. doi:10.2174/157339508784325046.
58. Taylor VH, Macqueen GM. The role of adipokines in understanding the associations between obesity and depression. *J Obes.* 2010; **2010**:748048. doi:10.1155/2010/748048.
59. Chehab FF. Leptin as a regulator of adipose mass and reproduction. *Trends Pharmacol Sci.* 2000; **21**(8):309–314. doi:10.1016/s0165-6147(00)01514-5.
60. Jéquier E. Leptin signaling, adiposity, and energy balance. *Ann N Y Acad Sci.* 2002; **967**:379–388. doi:10.1111/j.1749-6632.2002.tb04293.x.
61. Giannaccini G, Betti L, Palego L, *et al.* Serotonin transporter (SERT) and translocator protein (TSPO) expression in the obese ob/ob mouse. *BMC Neurosci.* 2011; **12**:18. doi:10.1186/1471-2202-12-18.
62. Considine RV, Sinha MK, Heiman ML, *et al.* Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med.* 1996; **334**(5):292–295. doi:10.1056/NEJM199602013340503.
63. Heymsfield SB, Greenberg AS, Fujioka K, *et al.* Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *JAMA.* 1999; **282**(16):1568–1575. doi:10.1001/jama.282.16.1568.
64. Zhou Y, Rui L. Leptin signaling and leptin resistance. *Front Med.* 2013; **7**(2):207–222. doi:10.1007/s11684-013-0263-5.

65. Milaneschi Y, Lamers F, Bot M, Drent ML, Penninx BW. Leptin dysregulation is specifically associated with major depression with atypical features: evidence for a mechanism connecting obesity and depression. *Biol Psychiatry*. 2017;**81**(9):807–814. doi:10.1016/j.biopsych.2015.10.023.
66. Sinha R, Jastreboff AM. Stress as a common risk factor for obesity and addiction. *Biol Psychiatry*. 2013;**73**(9):827–835. doi:10.1016/j.biopsych.2013.01.032.
67. Tomiyama AJ. Stress and obesity. *Annu Rev Psychol*. 2019;**70**:703–718. doi:10.1146/annurev-psych-010418-102936.
68. Pratt WE, Schall MA, Choi E. Selective serotonin receptor stimulation of the medial nucleus accumbens differentially affects appetitive motivation for food on a progressive ratio schedule of reinforcement. *Neurosci Lett*. 2012;**511**(2):84–88. doi:10.1016/j.neulet.2012.01.038.
69. Ohta Y, Kosaka Y, Kishimoto N, *et al.* Convergence of the insulin and serotonin programs in the pancreatic β -cell. *Diabetes*. 2011;**60**(12):3208–3216. doi:10.2337/db10-1192.
70. Paulmann N, Grohmann M, Voigt JP, *et al.* Intracellular serotonin modulates insulin secretion from pancreatic beta-cells by protein serotonylation. *PLoS Biol*. 2009;**7**(10):e1000229. doi:10.1371/journal.pbio.1000229.
71. Webb M, Davies M, Ashra N, *et al.* The association between depressive symptoms and insulin resistance, inflammation and adiposity in men and women. *PLoS One*. 2017;**12**(11):e0187448. doi:10.1371/journal.pone.0187448.
72. Lu XY. The leptin hypothesis of depression: a potential link between mood disorders and obesity? *Curr Opin Pharmacol*. 2007;**7**(6):648–652. doi:10.1016/j.coph.2007.10.010.
73. Yabut JM, Crane JD, Green AE, Keating DJ, Khan WI, Steinberg GR. Emerging roles for serotonin in regulating metabolism: new implications for an ancient molecule. *Endocr Rev*. 2019;**40**(4):1092–1107. doi:10.1210/er.2018-00283.
74. Cataldo Bascuñan LR, Lyons C, Bennet H, Artner I, Fex M. Serotonergic regulation of insulin secretion. *Acta Physiol (Oxf)*. 2019;**225**(1):e13101. doi:10.1111/apha.13101.