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Association of Genetic Variation in Cannabinoid Mechanisms and Gastric Motor Functions and Satiation in Overweight and Obesity

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Abstract

Objective—The endocannabinoid system is associated with food intake. Genes regulating cannabinoids are associated with obesity.

Hypothesis—Genetic variations in fatty acid amide hydroxylase (*FAAH*), and cannabinoid receptor 1 (*CNRI*) are associated with satiation and gastric motor function.

Methods—In 62 overweight or obese adults of European ancestry, single nucleotide polymorphisms (SNPs) of rs806378 (nearest gene *CNRI*) and rs324420 (nearest gene *FAAH*) were genotyped and the associations with gastric emptying (GE) of solids and liquids, gastric volume (GV) and satiation (maximum tolerated volume [MTV] and symptoms after Ensure® nutrient drink test) were explored using a dominant genetic model, with gender and BMI as co-variables.

Results—rs806378 CC genotype was associated with reduced fasting GV (210.2±11.0 mL for CC group compared to 242.5±11.3 mL for CT/TT group, p=0.031) and a modest, non-significant association with GE of solids (p=0.17). rs324420 genotype was not associated with alterations in gastric motor functions; however there was a difference in the Ensure® MTV (1174.6±37.2 mL for CC group compared to 1395.0±123.1 mL for CA/AA group, p= 0.046) suggesting higher satiation with CC genotype.

Conclusion—Our data suggest that *CNRI* and *FAAH* are associated with altered gastric functions or satiation that may predispose to obesity.

Keywords

gastric emptying; gastric volume; endocannabinoids; cannabinoid receptor; *FAAH*; *CNRI*

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Food intake and associated behavioral factors are important determinants of weight status (1), and the stomach signals satiation during feeding in response to the volume and calories ingested (2,3). Previous studies in our laboratory showed that the postprandial change in gastric volume is an independent predictor of maximum tolerated volume and satiation (4); in addition, when we applied pharmacological perturbations with i.v. erythromycin and atropine to induce postprandial discomfort including fullness and bloating, we observed in a multivariate analysis that accelerated gastric emptying of solids at 1 hour, and delayed gastric emptying at 4 hours, as well as fasting gastric volume were associated with postprandial symptom scores reflecting satiation (5,6). Postprandial gastric volume and change in gastric volume during fasting were univariately associated, but was not significant if fasting gastric volume was included in the model.

The endocannabinoid system, a lipid-derived signaling system, has central and peripheral physiologic functions (7). The endocannabinoids, anandamide and 2-arachidonyl glycerol (2-AG), bind to the cannabinoid (CB) receptor and regulate appetite. The cannabinoid receptor type 1 (CB1) is located centrally in the hypothalamus and nucleus accumbens and in peripheral tissues (8,9). There is evidence that gastric emptying is physiologically regulated in rodents by the endocannabinoid system, which is down regulated following a high fat diet leading to overweight (10). In a pharmacodynamic study in healthy human volunteers, we showed that the non-selective cannabinoid agonist dronabinol retards gastric emptying in females and increases fasting gastric volumes in males (11).

The endocannabinoid anandamide is hydrolyzed by the enzyme fatty acid amide hydrolase (FAAH), and the polymorphism rs324420 (*FAAH* C385A, located on chromosome 1 p35–p34) results in an amino acid change at position 129, P129T. This produces a variant that displays normal catalytic properties but an enhanced sensitivity to proteolytic degradation (12). Both cannabinoid receptor 1 (*CNR1*, located on 6q14–15) and *FAAH* play an integral part in the control of the endocannabinoid system and the response to endocannabinoids; these mechanisms also play a role in the development of obesity (12,13). Cannabinoids also modulate motor activity in the gastrointestinal tract; for example, non-selective cannabinoid agonist dronabinol inhibits gastric and colonic motility (11,14,15). In humans, studies using a CB1 receptor antagonist, rimonabant, have shown modest weight reduction and improvement in cardiovascular risk factors in obese subjects (16,17).

Common variations in genes controlling cannabinoid function or metabolism have been associated with the development of obesity, diabetes as well as with potential alterations in gastric function. *FAAH* rs324420 results in a mutant form of *FAAH* with reduced expression and cellular stability (18). Sipe and colleagues suggested that the *FAAH* rs324420 polymorphism is associated with overweight and obese individuals (19). Sipe et al. also reported that, in severe obesity (BMI>40kg/m²), significantly increased levels of the endocannabinoid anandamide and related N-acylethanolamines were documented in carriers of the *FAAH* 385 A mutant alleles compared with wild-type *FAAH* controls (20). This suggests that *FAAH* 385 A mutant alleles have a direct effect on elevated plasma endocannabinoid levels which may be biomarkers of risk for severe obesity (20). In addition, there is evidence that this missense mutation influences weight loss in response to a hypocaloric diet (21).

Polymorphisms in *CNR1* are also associated with obesity. Thus, *CNR1* gene variation rs203239 has been shown to predispose to weight gain in Europeans (22), rs1049353 (G1359A) is associated with fat distribution (23) and abdominal adiposity in men (24), and T allele carriers for the *CNR1* polymorphism rs806378 (C4558T) have increased odds ratio of childhood obesity (22) and experience increased antipsychotic-induced weight gain compared to CC carriers (25). rs1049353 is a synonymous change that does not alter the

amino acid. The T allele associated with *CNR1* polymorphism rs806378 is associated with altered nuclear protein binding in a functional (electron shift mobility) assay, suggesting it is a functional polymorphism (25). Therefore, we focused our study on the *CNR1* rs806378 polymorphism and studied its association with gastric function and satiation.

The aim of this study was to assess the association of allelic variations in the rs324420 (nearest gene *FAAH*) and rs806378 (nearest gene *CNR1*) single nucleotide polymorphisms (SNPs) with gastric motor and sensory functions, and satiation (that could predispose to the development of obesity) in a cohort of overweight and obese human participants.

MATERIALS AND METHODS

Participants

Selection of the 62 Caucasian participants has been thoroughly described elsewhere (26), and the majority were overweight or obese: normal weight (NW, n= 5; 49±6.5 years; 80% female; BMI 24.0±0.5 kg/m²); overweight (OW, n=28; 41±1.7 years; 79% female; BMI 28.1±0.3 kg/m²); and obese (Ob, n=29; 47±2.4 years; 59% female; BMI 34.9±0.6 kg/m²). They had previously enrolled in studies of the pharmacogenetics of sibutramine (27) and of gastric sensorimotor functions in obesity (4,28). We obtained approval to use stored DNA by the Mayo Clinic Institutional Review Board, in accordance with written informed consent from the participants in prior studies to use their stored DNA for research on obesity. One DNA sample was insufficient for assay; therefore the current study is based on 61 men and women between the ages of 18 and 65 years residing in Olmsted County, Minnesota. Exclusion criteria were a positive history of any systemic disease that could affect gastrointestinal motility; and use of medications that alter gastrointestinal motility, appetite, or absorption (e.g., orlistat). Permissible medications were multivitamins, birth control pills, estrogen, and thyroxine replacement, all at stable doses for at least 30 days prior to the studies. Women of childbearing potential had a negative pregnancy test within 48 hours before any radioisotopes were administered.

Experimental Protocol

The study was approved by the Mayo Clinic Institutional Review Board, and all participants gave written informed consent. On different days, they presented to the Mayo Clinic Clinical Research Unit at 7 a.m. after an 8 hour fasting period and underwent a gastric emptying scintigraphic study, a nutrient drink satiation test, and a gastric accommodation SPECT (single photon emission computed tomography) study, in this order (26). Gastric emptying and SPECT studies were performed at least 72 hours apart to avoid interference by ¹¹¹In from the meal ingested during the gastric emptying study with the measurement of gastric volume by SPECT.

Gastric Emptying Study with Scintigraphy

Participants ingested a solid and liquid caloric meal (total calories: 296 kcal, 32% protein, 35% fat, 33% carbohydrate) in which both phases of the meal were radiolabeled: 1.0 mCi ^{99m}Tc (technetium)-sulfur colloid was added to two raw eggs during the scrambling and cooking process. The scrambled eggs were served on one slice of buttered bread with 240 ml of 1% milk labeled with 0.1 mCi ¹¹¹In (indium)-DTPA. Anterior and posterior gamma camera images were obtained immediately after radiolabeled meal ingestion, every 15 minutes for the first 2 hours, then every 30 minutes for the next 2 hours [total 4 hours after the radiolabeled meal (4)]. Data were analyzed as in previous studies (29). Geometric mean of counts in gastric regions of interest on anterior and posterior images were used (after correction for radioisotope decay) to estimate the proportion of ^{99m}Tc or ¹¹¹In emptied

from the stomach at each time point. Gastric emptying $t_{1/2}$ for solids and liquids were estimated by linear interpolation of the data at each time point.

Gastric Volume and Accommodation Assessment with ^{99m}Tc -SPECT

We measured gastric volume during fasting and during 30 minutes after 300 mL of Ensure® (316 Kcal) using noninvasive single photon emission computed tomography. The method has been previously validated (30). Intravenous injection of ^{99m}Tc sodium pertechnetate, which is taken up by the parietal and non-parietal cells of the gastric mucosa, allows visualization of the stomach wall. Tomographic images of the gastric wall are obtained throughout the long axis of the stomach using a dual-head gamma camera (SMV SPECT System*, Twinsburg, Ohio, U.S.A.) that rotates around the body. The radiolabeled circumference of the gastric wall (rather than the intra-gastric content) is thus identified. Using the AVW 3.0 (Biomedical Imaging, Mayo Foundation) image processing libraries, a three-dimensional rendering of the stomach is obtained and its volume (mL) calculated. There is high intra-observer reproducibility to measure gastric volume with this technique (31). The inter-individual coefficient of variation (COVINTER) for all subjects in a study from our laboratory (n=433) was 32.6% fasting, 16.0% fed, and 19.0 % Δ fed - fasting. The intraindividual COV (COVINTRA) for 47 subjects with repeat estimates of gastric volume was 37.0% fasting, 17.6% fed, and 22.0 % Δ fed - fasting. COV_{INTRA} was stable over a time interval from 2 to 60 months (32). There are no significant differences by gender.

Nutrient Drink Satiation Test

We used a validated nutrient drink test (33) to measure satiation and postprandial symptoms when drinking a liquid nutrient at constant rate of 120ml every 4 minutes (Ensure®: 1 Kcal/mL, 11% fat, 73% carbohydrate and 16% protein). Participants scored the time needed to reach each level of fullness using a digital timer. Every 5 minutes, participants scored level of fullness or satiation using a scale combining verbal descriptors and numbers (0 = no symptoms; 5 = maximum or unbearable fullness/satiation). Nutrient intake was stopped when subjects reached the score of 5, with maximum satiation indicated by the maximum tolerated volume of Ensure®. Postprandial symptoms of fullness, nausea, bloating and pain, were measured 30 minutes after the meal using 100mm horizontal visual analog scales (VAS), with the words 'none' and 'worst ever' anchored at the left and right ends of the lines for each symptom.

Determination of Genotypes

DNA was extracted from whole blood as previously described. Genotyping of *FAAH* (rs324420), and *CNR1* (rs806378) was performed using Taqman™ SNP Genotyping assays (Applied Biosystems Inc., Foster City, CA) in accordance with the manufacturer's instructions.

Statistical Analysis

All data are presented as means $\pm$ SEM, or percentages, as noted. The univariate associations of subject characteristics and response measures (e.g., gastric emptying $T_{1/2}$ values) with overall genotype were assessed using Fisher's Exact test (e.g. association with categorical variables like gender) and for quantitative traits, the Kruskal-Wallis test assuming a general allelic model for three genotypes and pair-wise associations using the Wilcoxon rank sum test based on the dominant genetic model (i.e. pooling the minor allele homozygote with the heterozygote genotype: TT plus CT versus CC for the rs806378 (*CNR1*) genotypes, and CA and AA versus CC for the rs324420 (*FAAH*) genotype. This grouping is based on prior demonstration that the disease-predisposing allele is the A allele for the rs324420 (*FAAH*) genotype and the T allele for the rs806378 (*CNR1*) genotype. This

assumes the single allele has a biological effect. The association of genotype with physiologic response measures were also assessed using an analysis of covariance adjusting for gender and BMI.

A sample size assessment for detecting clinically relevant associations for the rs806378 (*CNR1*) and rs324420 (*FAAH*) genotypes was done by estimating the differences between 2 groups [i.e. assuming a dominant genetic model with CC vs. CT/TT for rs806378 (*CNR1*) and CC vs. CA/AA for rs324420 (*FAAH*)] that could be detected given the observed variation in the measured responses and the number of subjects that were obtained in each genotype group (27 CC vs. 35 CT/TT for rs806378 and 49 CC vs. 12 CA/AA for rs324420). The differences between groups that could be detected with approximately 80% power (two-sided α level of 0.05) using a two-sample t-test [assuming the listed pooled standard deviation (SD)] are listed in Table 1. Except for gastric emptying (two endpoints), each physiologic response corresponds to a distinct null hypothesis (e.g. association of genotype of interest with nutrient drink test maximum tolerated volume, i.e. satiation). Therefore, we did not correct for assessing associations with multiple intermediate phenotypes. A *p*-value of less than 0.05 was considered to be statistically significant except in the assessment of the association between genotype and gastric emptying (α level of 0.025 in view of two comparisons for solids and liquids). Analyses used the SAS® statistical package.

RESULTS

Subject Characteristics

Demographic characteristics of individuals in each genotype group are outlined in Table 2. There were no significant overall associations of any genotype with age, gender, BMI or weight.

Relationship of rs806378 Genotype with Gastric Motor Functions and Satiation

rs806378 genotype (nearest gene *CNR1*) was associated with fasting gastric volume ($p=0.05$ by the general allelic model, and $p=0.031$ by the dominant genetic model, ANCOVA adjusting for gender and BMI). The CC genotype was associated with a smaller gastric volume compared with the TT genotype participants. There was no significant association with postprandial gastric volume or delta volume from fasting that represents gastric accommodation.

Patients with rs806378 CC genotype had numerically non-significant effect on gastric emptying of solids with a mean difference in gastric emptying $T_{1/2}$ of 18 minutes ($p=0.17$). There was no significant association between rs806378 genotype with gastric emptying of liquids, and satiation indices [MTV ($p=0.534$) and aggregate score ($p=0.098$)].

Relationship of rs324420 Genotype with Gastric Motor Functions and Satiation

There was no significant association of rs324420 genotype (nearest gene *FAAH*) with solid and liquid gastric emptying, fasting gastric volume or aggregate symptom score. Similarly, there was no significant association with postprandial gastric volume or (delta) accommodation volume from fasting. However, there was a significant numerical difference in mean MTV based on the rs324420 genotype. Thus, the MTV with the CC genotype being ~220mL (~235kcal) lower than in the CA/AA genotype group (Table 3; univariate association by Wilcoxon rank sum test, $p=0.051$). Similarly, analysis by ANCOVA model (with BMI and sex as covariates) was significant ($p=0.046$), based on comparison of the following least square means and 95%CI: CC genotype 1224 (1135,1313) mL compared to CA/AA genotype 1414 (1247,1581) mL.

DISCUSSION

Genes involved in endocannabinoid metabolism (*FAAH*), cannabinoid 1 receptor function (*CNR1*) may be associated with obesity. In this study, we explored the association of these genes with gastric motor function and satiation in humans. rs806378 genotype (nearest gene *CNR1*) influenced fasting gastric volume which is a significant factor in the genesis of postprandial symptoms such as fullness and bloating. In this study, *CNR1* genotype was not associated with satiation. In contrast, rs324420 (nearest gene *FAAH*) genotype did not influence gastric motor functions, but there was a substantially lower maximum tolerated volume in CC carriers of the rs324420 SNP compared to CA/AA carriers.

Evidence suggests that gastric sensorimotor functions play a role in the development of obesity (4–6). Satiation, mediated peripherally by the stomach, determines the amount of food intake and there is growing evidence to suggest that there may be a genetic susceptibility to postprandial satiation (26). Individuals with the CC genotype for the *CNR1* polymorphism had decreased fasting gastric volume compared to the TC/TT individuals, but there was no association of the *CNR1* polymorphism with postprandial satiation or gastric motor functions. The apparently modest, but statistically significant difference in the fasting gastric volumes between the *CNR1* genotype groups is nevertheless relevant. Using the data we previously published elsewhere using identical methods (6), we have applied the observed relationship of 7.23kcal of Ensure® per mL of fasting gastric volume. Thus, the apparently modest absolute difference of 32mL (which is equivalent to ~15% difference in the fasting gastric volume) in the *CNR1* CC versus CT/TT genotype would be expected to result in a difference in meal ingested of a ~230kcal. This could have a significant effect on satiation and weight gain.

The weight of evidence indicates that the major effects of cannabinoids on food intake are through central effects on the CB1 receptors. This has been shown in animal studies where administration of a CB1 agonist directly into the brain elicits food intake and the inverse is seen after administration of a CB1 antagonist (34–36). In human studies, administration of a CB1 receptor antagonist, rimonabant, showed significant weight reduction. However, this benefit occurred at the expense of adverse effects such as depression, anxiety, and nausea, demonstrating central effects of CB1 antagonism and precluding the use of rimonabant and taranabant as anti-obesity medications (37,38). Recent development of the peripherally active, CB1 receptor agonist, AM841, suggests that a new class of agents did not have central effects in rats and may be tested in humans in the future (39).

On the other hand, there may be a peripheral component to satiation (reduced fasting gastric volume) that is associated with variation in *CNR1*. In prior studies where gastric functions in obese subjects had been pharmacologically manipulated to simulate dyspepsia, fasting gastric volume was associated with increased satiation measured by symptom scores^{5,6}. While we did not find a significant association ($p=0.098$) between *CNR1* polymorphism and aggregate symptom scores, this may be due to a type II error, as symptoms scores tend to be more variable compared to the tight coefficient of variation observed with the intermediate phenotype of gastric volume. There are other studies that support a potential effect of cannabinoids on gastric emptying which may also influence satiation. Thus, mice fed a standard diet and receiving anandamide had delayed gastric emptying, an effect that was abolished with the CB1 receptor antagonist, rimonabant. In addition, the non-selective cannabinoid agonist dronabinol retarded gastric emptying in humans (22). These data are consistent with the notion that CB1 receptors expressed peripherally on vagal nerves transmit satiation signals from the GI tract to the brain or reduce afferent signals that determine the gastric emptying rate (40,41). These observations suggest that more selective

and restrictive peripheral modulators of the CB1 receptor may still have potential in the treatment of obesity.

Genetic variation in rs324420 (tagging the *FAAH* gene) is associated with satiation with a lower maximum tolerated volume of Ensure® nutrient drink in CC carriers. Our results are consistent with the observation of Sipe et al. that subjects with the *FAAH* 385 A mutant alleles (controlling for BMI) had significant elevation of plasma anandamide and related N-acylethanolamines (20). Thus, since the A allele results in a variant of *FAAH* that displays normal catalytic properties but an enhanced sensitivity to proteolytic degradation (12), their *FAAH* enzyme is less effective and, as a result, endocannabinoids would be metabolized less in the A allele carriers, and their plasma levels are higher. In contrast, the CC genotype group would have more effective endocannabinoid degradation resulting in lower appetite and greater satiation (lower MTV by about 190mL or 200 kcal during the one meal, even after correcting for BMI) in our cohort.

In summary, our data suggest that genetic variation in *CNRI*, but not *FAAH*, is associated with variations in gastric functions that may predispose to obesity. On the other hand, *FAAH* alters satiation (MTV). Further studies exploring the role of genetic susceptibility in mechanisms to alter gastric functions and predispose to obesity are warranted. Moreover, these genotype-intermediate phenotype association studies may help identify targets for future anti-obesity clinical trials.

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REFERENCES

1. Blundell JE, Gillett A. Control of food intake in the obese. *Obesity Research*. 2001; 9(Suppl 4): 263S–270S. [PubMed: 11707552]
2. Deutsch JA. The role of the stomach in eating. *American Journal of Clinical Nutrition*. 42:1040–1043. 1985.
3. Deutsch JA, Young WG, Kalogeris TJ. The stomach signals satiety. *Science*. 1978; 201:165–167. [PubMed: 663647]
4. Vazquez Roque MI, Camilleri M, Stephens DA, et al. Gastric sensorimotor functions and hormone profile in normal weight, overweight, and obese people. *Gastroenterology*. 2006; 131:1717–1724. [PubMed: 17087952]
5. Delgado-Aros S, Camilleri M, Castillo EJ, et al. Effect of gastric volume or emptying on meal-related symptoms after liquid nutrients in obesity: a pharmacologic study. *Clinical Gastroenterology and Hepatology*. 2005; 3:997–1006. [PubMed: 16234046]
6. Delgado-Aros S, Cremonini F, Castillo JE, et al. Independent influences of body mass and gastric volumes on satiation in humans. *Gastroenterology*. 2004; 126:432–440. [PubMed: 14762780]
7. Izzo AA, Sharkey KA. Cannabinoids and the gut: new developments and emerging concepts. *Pharmacol Ther*. 2010; 126:21–38. [PubMed: 20117132]
8. Kirkham TC. Endocannabinoids in the regulation of appetite and body weight. *Behav Pharmacol*. 2005; 16:297–313. [PubMed: 16148436]
9. Izzo AA, Camilleri M. Emerging role of cannabinoids in gastrointestinal and liver diseases: basic and clinical aspects. *Gut*. 2008; 57:1140–55. [PubMed: 18397936]
10. Di Marzo V, Capasso R, Matias I, Aviello G, Petrosino S, Borrelli F, Romano B, Orlando P, Capasso F, Izzo AA. The role of endocannabinoids in the regulation of gastric emptying: alterations in mice fed a high-fat diet. *Br J Pharmacol*. 2008; 153:1272–1280. [PubMed: 18223666]

11. Esfandyari T, Camilleri M, Ferber I, Burton D, Baxter K, Zinsmeister AR. Effect of a cannabinoid agonist on gastrointestinal transit and postprandial satiation in healthy human subjects: a randomized, placebo-controlled study. *Neurogastroenterol Motil.* 2006; 18:831–838. [PubMed: 16918762]
12. de Kloet AD, Woods SC. Minireview: Endocannabinoids and their receptors as targets for obesity therapy. *Endocrinology.* 2009; 150:2531–2536. [PubMed: 19372200]
13. Osei-Hyiaman D, DePetrillo M, Pacher P, et al. Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J Clin Invest.* 2005; 115:1298–1305. [PubMed: 15864349]
14. Camilleri M, Carlson P, McKinzie S, et al. Genetic variation in endocannabinoid metabolism, gastrointestinal motility, and sensation. *Am J Physiol.* 2008; 294:G13–G19.
15. Esfandyari T, Camilleri M, Busciglio I, Burton D, Baxter K, Zinsmeister AR. Effects of a cannabinoid receptor agonist on colonic motor and sensory functions in humans: a randomized, placebo-controlled study. *Am J Physiol.* 2007; 293:G137–G145.
16. Pi-Sunyer FX, Aronne LJ, Heshmati HM, et al. Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients: RIO-North America: a randomized controlled trial. *JAMA.* 2006; 295:761–775. [PubMed: 16478899]
17. Van Gaal LF, Rissanen AM, Scheen AJ, et al. Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *Lancet.* 2005; 365:1389–1397. [PubMed: 15836887]
18. Chiang KP, Gerber AL, Sipe JC, Cravatt BF. Reduced cellular expression and activity of the P129T mutant of human fatty acid amide hydrolase: evidence for a link between defects in the endocannabinoid system and problem drug use. *Hum Mol Genet.* 2004; 13:2113–9. [PubMed: 15254019]
19. Sipe JC, Waalen J, Gerber A, et al. Overweight and obesity associated with a missense polymorphism in fatty acid amide hydrolase (FAAH). *Int J Obes (Lond).* 2005; 29:755–759. [PubMed: 15809662]
20. Sipe JC, Scott TM, Murray S, Harismendy O, Simon GM, Cravatt BF, Waalen J. Biomarkers of endocannabinoid system activation in severe obesity. *PLoS One.* 2010; 5:e8792. [PubMed: 20098695]
21. de Luis DA, Gonzalez Sagrado M, Aller R, Izaola O, Conde R. Effects of C358A missense polymorphism of the endocannabinoid degrading enzyme fatty acid amide hydrolase on weight loss after a hypocaloric diet. *Metabolism.* Aug 16.2010 [Epub ahead of print]. doi:10.1016/j.metabol.2010.07.007.
22. Benzinou M, Chèvre JC, Ward KJ, Lecoœur C, Dina C, Lobbens S, Durand E, Delplanque J, Horber FF, Heude B, Balkau B, Borch-Johnsen K, Jørgensen T, Hansen T, Pedersen O, Meyre D, Froguel P. Endocannabinoid receptor 1 gene variations increase risk for obesity and modulate body mass index in European populations. *Hum Mol Genet.* 2008; 17:1916–21. [PubMed: 18375449]
23. Frost M, Nielsen TL, Wraae K, Hagen C, Pters E, Beckers S, De Freitas F, Brixen K, Van Hul W, Andersen M. Polymorphisms in the endocannabinoid receptor 1 in relation to fat mass distribution. *Eur J Endocrinol.* 2010; 163:407–12. [PubMed: 20587580]
24. Peeters A, Beckers S, Mertens I, Van Hul W, Van Gaal L. The G1422A variant of the cannabinoid receptor gene (CNR1) is associated with abdominal adiposity in obese men. *Endocrine.* 2007; 31:138–41. [PubMed: 17873324]
25. Tiwari AK, Zai CC, Likhodi O, et al. A common polymorphism in the cannabinoid receptor 1 (CNR1) gene is associated with antipsychotic-induced weight gain in Schizophrenia. *Neuropsychopharmacology.* 2010; 35:1315–1324. [PubMed: 20107430]
26. Papathanasopoulos A, Camilleri M, Carlson P, Vella A, Linker Nord S, Burton D, Odunsi S, Zinsmeister AR. A preliminary candidate genotype-intermediate phenotype study of satiation and gastric motor function in obesity. *Obesity.* 2010; 18:1201–1211. [PubMed: 19876010]
27. Grudell AB, Sweetser S, Camilleri M, et al. A controlled pharmacogenetic trial of sibutramine on weight loss and body composition in obese or overweight adults. *Gastroenterology.* 2008; 135:1142–1154. [PubMed: 18725220]

28. Grudell AB, Camilleri M, Carlson P, et al. An exploratory study of the association of adrenergic and serotonergic genotype and gastrointestinal motor functions. *Neurogastroenterol Motil.* 2008; 20:213–219. [PubMed: 17971028]
29. Cremonini F, Mullan BP, Camilleri M, Burton DD, Rank MR. Performance characteristics of scintigraphic transit measurements for studies of experimental therapies. *Aliment Pharmacol Ther.* 2002; 16:1781–1790. [PubMed: 12269971]
30. Bouras EP, Delgado-Aros S, Camilleri M, et al. SPECT imaging of the stomach: comparison with barostat, and effects of sex, age, body mass index, and fundoplication. Single photon emission computed tomography. *Gut.* 2002; 51:781–786. [PubMed: 12427776]
31. De Schepper H, Camilleri M, Cremonini F, Foxx-Orenstein A, Burton D. Comparison of gastric volumes in response to isocaloric liquid and mixed meals in humans. *Neurogastroenterol Motil.* 2004; 16:567–573. [PubMed: 15500513]
32. Breen M, Camilleri M, Burton D, Zinsmeister AR. Performance characteristics of the measurement of gastric volume using single photon emission computed tomography. *Neurogastroenterol Motil.* 2010 in press.
33. Chial HJ, Camilleri C, Delgado-Aros S, et al. A nutrient drink test to assess maximum tolerated volume and postprandial symptoms: effects of gender, body mass index and age in health. *Neurogastroenterol Motil.* 2002; 14:249–253. [PubMed: 12061909]
34. Williams CM, Kirkham TC. Observational analysis of feeding induced by Delta9-THC and anandamide. *Physiol Behav.* 2002; 76:241–250. [PubMed: 12044596]
35. Kirkham TC, Williams CM, Fezza F, Di Marzo V. Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. *Br J Pharmacol.* 2002; 136:550–557. [PubMed: 12055133]
36. Verty AN, McGregor IS, Mallet PE. Paraventricular hypothalamic CB(1) cannabinoid receptors are involved in the feeding stimulatory effects of Delta(9)-tetrahydrocannabinol. *Neuropharmacology.* 2005; 49:1101–1109. [PubMed: 16098995]
37. Despres JP, Golay A, Sjostrom L. Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. *N Engl J Med.* 2005; 353:2121–2134. [PubMed: 16291982]
38. Aronne LJ, Tonstad S, Moreno M, et al. A clinical trial assessing the safety and efficacy of taranabant, a CB1R inverse agonist, in obese and overweight patients: a high-dose study. *Int J Obes (Lond).* 2010; 34:919–935. [PubMed: 20157323]
39. Abalo R, Chen C, Vera G, Castillo MA, Fichna J, Thakur GA, Makriyannis A, Martin MI, Storr M. The cannabinoid (CB1) agonist AM841 slows GI motility in the rat by peripheral actions. *Neurogastroenterol Motil.* 2010; 22(Suppl 1):69.
40. Burdyga G, Lal S, Varro A, Dimaline R, Thompson DG, Dockray GJ. Expression of cannabinoid CB1 receptors by vagal afferent neurons is inhibited by cholecystokinin. *J Neurosci.* 2004; 24:2708–2715. [PubMed: 15028763]
41. Di Marzo V, Capasso R, Matias I, et al. The role of endocannabinoids in the regulation of gastric emptying: alterations in mice fed a high-fat diet. *Br J Pharmacol.* 2008; 153:1272–1280. [PubMed: 18223666]

Table 1
Sample Size Assessment

The table shows differences between two *CNR1* or *FAAH* genotype groups that could have been detected with approximately 80% power (two-sided $\alpha = 0.05$) using a two-sample t-test (assuming the observed pooled standard deviation [SD] listed in the table).

	SD	Mean (Group 1)	Mean (Group 2)
Fasting Gastric Volume, ml	64	230	277
Δ Postprandial Accommodation Volume, ml	85	490	553
Aggregate Symptom Score (maximum 400)	72	150	203
Maximum Tolerated Volume, ml	312	1210	1440
Gastric Emptying of Solids T ½, min	36	120	147
Gastric Emptying of Liquids T ½, min	9.7	22	30

Table 2

Subject Characteristics by Genotype (summary values are mean $\pm$ SEM, unless otherwise indicated)[†]

	FAAH				CNRI		
	CC	CA	AA		CC	CT	TT
N	49	11	1		27	28	7
Females, %	71.4	54.5	100		77.7	60.7	71.4
Age, Years	45.2 $\pm$ 1.6	41.5 $\pm$ 2.9	22.0		46 $\pm$ 2.4	45.0 $\pm$ 2.1	36.3 $\pm$ 2.9
Weight, Kg	86.7 $\pm$ 2.0	95.2 $\pm$ 5.3	77.7		91.0 $\pm$ 2.5	85.0 $\pm$ 3.4	84.2 $\pm$ 4.1
BMI, Kg/m ²	31.0 $\pm$ 0.6	31.7 $\pm$ 1.7	27.9		32.7 $\pm$ 2.5	29.7 $\pm$ 0.9	28.9 $\pm$ 1.1

[†] Fisher's Exact test applied for gender; Kruskal-Wallis test for age, weight and BMI

Table 3

Gastric Motor and Sensory Functions Values by the Dominant Genetic Model

Genotype	FAAH		CNR1	
	CC	CA/AA	CC	CT/TT
N	49	12	27	35
Gastric emptying, solids, $t_{1/2}$, min	122.8±5.5	108.0±9.4	129.8±8.4	111.6±5.1
Gastric emptying, liquids, $t_{1/2}$, min	22.2±1.5	18.5±2.7	21.9±2.2	21.3±1.6
Fasting gastric volume, mL	225.4±7.7	243.8±29.5	210.2±11.0*	242.5±11.3
Δ Postprandial accommodation volume, ml	483.2±12.3	505.6±29.0	488.7±17.5	486.2±15.1
Maximum tolerated volume mL	1174.6±37.2	1395.0±123.1 [#]	1221.2±58.3	1214.2±54.0
Aggregate Symptom Score, mm	152.4±10.1	144.0±22.3	161.6±16.2	142.4±10.3

Data show mean ± SEM

*
p=0.031[#]
p=0.046 based on ANCOVA model (with BMI and gender as co-variables)