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Brief Report

# Differential regulation of metabolic parameters by energy deficit and hunger

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ABSTRACT

*Aims.* Hypocaloric diet decreases both energy expenditure (EE) and respiratory exchange rate (RER), affecting the efficacy of dieting inversely. Energy deficit and hunger may be modulated separately both in human and animal studies by drug treatment or food restriction. Thus it is important to separate the effects of energy deficit and hunger on EE and RER.

*Methods.* Three parallel and analogous experiments were performed using three pharmacologically distinct anorectic drugs: rimonabant, sibutramine and tramadol. Metabolic parameters of vehicle- and drug-treated and pair-fed diet-induced obese mice from the three experiments underwent common statistical analysis to identify effects independent of the mechanisms of action. Diet-induced obesity (DIO) test of tramadol was also performed to examine its anti-obesity efficacy.

*Results.* RER was decreased similarly by drug treatments and paired feeding throughout the experiment irrespective of the cause of reduced food intake. Contrarily, during the passive phase, EE was decreased more by paired feeding than by both vehicle and drug treatment irrespective of the drug used. In the active phase, EE was influenced by the pharmacological mechanisms of action. Tramadol decreased body weight in the DIO test.

*Conclusions.* Our results suggest that RER is mainly affected by the actual state of energy balance; conversely, EE is rather influenced by hunger. Therefore, pharmacological medications that decrease hunger may enhance the efficacy of a hypocaloric diet by maintaining metabolic rate. Furthermore, our results yield the proposal that effects of anorectic drugs on EE and RER should be determined compared to vehicle and pair-fed groups, respectively, in animal models.



## 1. Introduction

Metabolic effects of various anti-obesity drugs compared to either *ad libitum* or pair-fed controls are widely described [1,2]. Although both drug-treated and pair-fed groups experience energy deficit, only paired feeding involves food restriction, i.e. prolonged or increased hunger. Human data suggest a possible relation between hunger and adaptive reduction of thermogenesis during a hypocaloric diet [1,3]. This leaves open the possibility of there being differences between the metabolic consequences of drug- and restriction-induced underfeeding.

Low-calorie diet decreases both energy expenditure (EE) and respiratory exchange rate (RER) [4–6]. These processes affect the outcome of a hypocaloric diet targeting weight loss oppositely:

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decreased RER is a marker of increased fat catabolism [7,8], while reduced EE decreases the efficacy of dieting [9,10].

In order to separate the specific effects of certain drugs from their general anorectic effect, we have chosen three compounds with fundamentally different mechanisms of action (MoA-s): rimonabant (cannabinoid CB1 receptor antagonist), sibutramine (an amphetamine derivate, serotonin and noradrenaline reuptake inhibitor [11]) and tramadol (μ-opioid receptor agonist [12]).

Both sibutramine and rimonabant decrease appetite in low doses and affect energy expenditure in higher doses [2,13–16]. Tramadol was recently reported to decrease food intake after surgery, but no vehicle group was used in that experiment [17].

Our aim was to test the hypothesis that there are fundamental differences between the metabolic effects of food restriction and drug-induced reduction in food intake, regardless of the exact pharmacological effect of a certain drug.

## 2. Methods

### 2.1. Drugs

Sibutramine hydrochloride (Jiangyin Eastern Medical Raw Materials, Jiangyin, China) and tramadol hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in distilled water; rimonabant (Bosche Scientific, New Brunswick, NJ, USA) was dissolved in 5% Tween 80 in distilled water because of solubility issues.

### 2.2. Animals

Male C57Bl6 mice (Harlan, Udine, Italy, 4 animals per cage) were kept on a 12:12 h light–dark cycle and fed with a high-fat diet (D12492, 60% of energy content from fat, Research Diets, New Brunswick, NJ, USA) throughout the whole process. After twelve weeks of fatting, 20-week-old animals were isolated and habituated to experimental circumstances. Oral treatments were performed before lights off. In case of tramadol, a second treatment was also performed six hours later in order to maintain the effective plasma level. Body weights were measured each day before the first treatment. All procedures had been approved by the local ethical committee and conformed to the national guidelines (decree No. 40/2013. (II. 14) of the Hungarian Government) and the directive 2010/63/EU of the European Parliament.

### 2.3. Indirect Calorimetry

Indirect calorimetry was performed using a 16-cage PhenoMaster system (TSE Systems, Bad Homburg, Germany). Three analogous experiments were performed dedicated to study the effects of sibutramine (3 mg/kg), tramadol (30 mg/kg per treatment) and rimonabant (3 mg/kg). Each experiment consisted of three separate experimental days (one day biweekly during the first, third and fifth week of the same 5-week-long period). Baseline measurements were performed before each experimental day. During each experimental day, each mouse was assigned to one of the three groups (vehicle, drug-treated, pair-fed) using the same randomized crossover design for the three experiments. Paired feeding was performed on an hourly basis. Every measurement started at lights off and lasted for 23 hours.

### 2.4. Statistical Analysis

Regarding the DIO test, body weights have been analyzed with one-way ANOVA (n = 8 per group).

For the indirect calorimetry (n = 16 per experiment), data were collected separately for the dark and light phases because we did not intend to compare the two phases by any means. RER and EE data for every 30-minute period were averaged for the given phases. Repeated measures ANOVA was performed with factors "group" (within-subject design) and "experiment" (between-subject design). Significant "group" effect suggested that there is a difference between vehicle, drug-treated and pair-fed groups. Significant "experiment" × "group" interaction suggested that this effect was influenced by which experiment they participated in, i.e. that effects differed between each drug. Contrarily, the absence of interaction suggested that the effects of drugs were identical. In case of significant "group" effect or "experiment" × "group" interaction, a one- or two-factor Tukey's HSD post hoc test was used, respectively.

Mauchly's sphericity test was used to validate repeated measures ANOVA tests. All statistical analyses have been carried out using STATISTICA 10 software (StatSoft, Tulsa, OK, USA).

## 3. Results

### 3.1. Diet-induced Obesity Test

Tramadol, administered 30 mg/kg twice daily decreased body weight significantly in dietary obese C57Bl/6 mice from day nine ($n = 8$, $F_{1,14} > 4.71$, $p < 0.05$, Fig. 1).

### 3.2. Indirect Calorimetry

After exclusions based on baseline data, animal numbers per experiment were n = 13 (rimonabant and sibutramine) or n = 12 (tramadol).

#### 3.2.1. Food Intake

ANOVA test revealed that the difference between treatment groups (active: $F_{2,70} = 51.173$, $p < 0.001$, passive: $F_{2,70} = 9.709$, $p < 0.001$) was identical between the experiments only in the active phase (active: $F_{4,70} = 0.942$, $p = 0.445$, passive: $F_{4,70} = 3.056$, $p = 0.022$), when drug treatments and paired feedings similarly decreased food intake. In the passive phase, only sibutramine and its pair-fed group showed decreased food intake compared to vehicle (Fig. 2/A).

#### 3.2.2. Respiratory Exchange Rate

For both phases, ANOVA tests revealed that the difference between treatment groups (active: $F_{2,70} = 38.759$, $p < 0.001$, passive: $F_{2,70} = 33.798$, $p < 0.001$) was identical between the experiments in both phases (active: $F_{4,70} = 0.247$, $p = 0.911$, passive: $F_{4,70} = 0.665$, $p = 0.619$). Drug treatment and paired feeding decreased RER in both phases (Fig. 2/B).

#### 3.2.3. Energy Expenditure

The difference between treatment groups (active: $F_{2,70} = 20.287$, $p < 0.001$, passive: $F_{2,70} = 20.949$, $p < 0.001$), was