Serum Metabolic Signature in an animal model of binge eating by Nuclear Magnetic Resonance Spectroscopy

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Target Audience: NMR Scientists, Psychiatrics and Psychologists, Nutritionist Scientists.

Purpose– Binge Eating (BE) episodes are characterized by uncontrollable, distressing eating of a large amount of highly palatable food (HPF). These episodes represent a central feature of bingeing related eating disorders, such as binge eating disorder, bulimia nervosa, and binge/purge subtype anorexia nervosa. Considerable evidence suggests that BE may be caused by a unique interaction between dieting and stress. In the model adopted by our group BE for HPF is evoked in rats by the combination of cyclic food restrictions and stress[1]. The model uses female rats in relation to the higher prevalence of BE disorders in women. Moreover, according to the inverse association between plasma estradiol levels and BE, we recently demonstrated that during the estrous phase, BE was not induced in our experimental conditions in female rats (Figure 1)[2]. In order to investigate Binge Eating behavior in female rats, for the first time, we analyzed the metabolic profile obtained from biological fluids (serum) of rats exposed or not to cycles of food restriction and a stressful challenge (respectively NR-NS and R-S group) through the Nuclear Magnetic Resonance Spectroscopy. The NMR spectra constitute a "fingerprint" of the NMR detectable part of the whole metabolome. The metabolomic research is a consolidated area aimed to detect the pool of metabolites in biological systems [3]. The metabolomic profile is due to low-molecular weight compounds that are products in various metabolic pathway. These small molecules include compounds such as lipids, sugar, aminoacids, nucleodides and a number of different organic molecules that are reactants, intermediates or products of biochemical reactions as well as building blocks for all other biochemical species including proteins, nucleic acids and cell membranes.

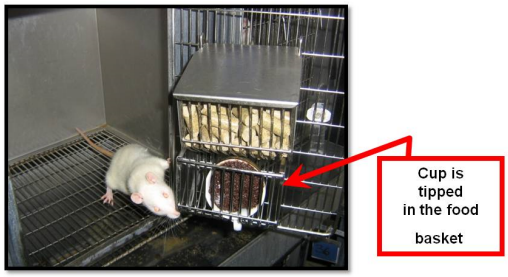

Figure 1: BE for HPF is evoked in rats by the combination of cyclic food restrictions and stress

Materials and Methods– *Animals.* Female Sprague-Dawley rats (Charles River, Calco, Como, Italy), 52-day-old at the beginning of the experiment, were used. Rats were acclimated to individual cages under a 12-h light/dark cycle. *Diets.* Animals were offered standard rat food pellets (4RF18, Mucedola, Settimo Milanese, Italy; 2.6 kcal/g) and a HPF. The HPF was a paste in texture, prepared by mixing: (a) 52 % Nutella (Ferrero, Alba (TO), Italy) chocolate cream (5.33 kcal/g; 56%, 31%, and 7% from carbohydrate, fat and protein, respectively), (b) 33 % grounded food pellets (4RF18), (c) 15 % water. *Stress.* Acute stress was elicited by exposing rats to HPF, but preventing them from access to it for 15 min, while rats were able to see and smell it. *Experimental procedure* Animals were divided in 2 groups of 10: 1. rats not food restricted and not exposed to stress **(NR + NS)**; 2. rats food restricted and exposed to stress **(R + S)**. They were submitted to three consecutive 8-day cycles followed by the final test on day 25 (**Table 1**): **(1) NR + NS** had chow ad libitum for 4 days; on days 5–6, they received chow ad libitum+HPF for 2 h; on days 7–8 they had chow ad libitum; on day 25, they were not exposed to stress;**(2) R + S** had food available like the R + NS group, and on day 25, they were exposed to stress. Animals were scarified immediately after the stress procedure. The estrous cycle phase determined by vaginal smear and serum collected for ^{1}H High Resolution Nuclear Magnetic Resonance (HR NMR) spectroscopy analyses. 300 ul serum of rats were used for the NMR analyses. We acquired one- and two- dimensional experiments from the two groups for the characterization of the metabolic profile. Three different types of 1D ^{1}H spectra were acquired by using: i) a composite pulse sequence (zgcppr), ii) a water-suppressed spin-echo Carr-Purcell-Meiboom-Gill (CPMG) sequence (cpmgpr), and iii) a sequence for diffusion measurements based on stimulated echo and bipolar-gradient pulses (ledbpgp2s1d).

Group	Days 1-4	Days 5-6	Days 7-8	Days 9-12	Days 13-14	Days 15-16	Days 17-20	Days 21-24	Days 25
NR-NS	Ad lib chow	Ad lib chow + HPF (2 h)	Ad lib chow	Ad lib chow	Ad lib chow + HPF (2 h)	Ad lib chow	Ad lib chow	Ad lib chow	No stress
R-S	Restricted chow to 66%	Ad lib chow + HPF (2 h)	Ad lib chow	Restricted chow to 66%	Ad lib chow + HPF (2 h)	Ad lib chow	Restricted chow to 66%	Ad lib chow	Stress

Table 1. The Schedule Adopted to Evoke Binge Eating in R+S respect to NR+NS group.

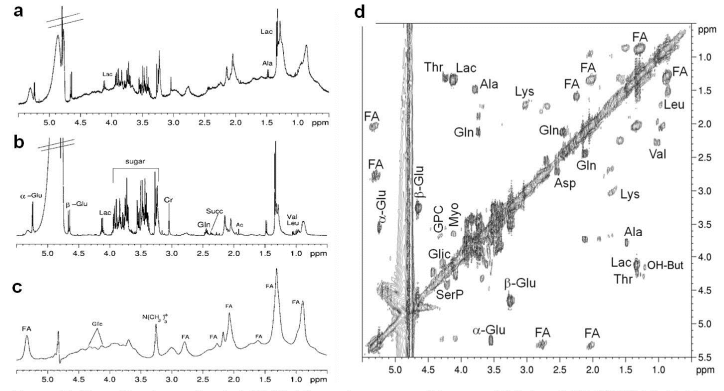

Figure 2: Convetional presaturated 1D ^{1}H (a) spectra: zgcppr. (b) cpmg, (c) led and 2D COSY (d). Lipids, macromolecules and small metabolites contributions are labelled (Ala: alanine; Ac: acetate; Lac: lactate; Gln: glutamine; Cr: creatine; Gly: glycine; Myo: myo-inositol; Succ: succinate; GPC: glycerophosphocholine; FA: fatty acid; Thr; threonine; Lys: lysine; α-Glc: α-glucose; β-Glc: β-glucose; Val: valine; Leu: leucine; $N(CH_3)_3$) phospholipids contribution.

Results– More than 30 metabolites were detected and assigned. After the metabolites characterization (Figure 2) of serum groups, we analyzed metabolomic NMR data: with a quantitative approache to better understand the differences between the two groups. On 1D ^{1}H CPMG experiments we performed quantification of metabolites. Peak areas were determined using Mnova software (MestReNova, ver. 8.1.0, 2012 Mestrelab Research S. L., Santiago de Compostela, Spain). *Statistical analysis.* Data were reported as means ± standard errors. P<0.05 was considered to indicate a statistically significant difference. (Figure 3) **Discussion-** Significant differences were found in small metabolites such as glutamine, lactate, and glycerolphosohorylcholine. Another important difference is in the amount of lipids more evident in the R+S animals model of BE compared to relative NR-NS animals. Lipids amount may be is related to the Adipocyte fatty acid binding protein (A-FABP), that has been suggested to play an important role in fat metabolism linking obesity and the metabolic syndrome. The variation in A-FABP plasma levels reflect alterations in nutritional status for example in patients with anorexia nervosa [4]. The results will be used for the study of innovative pharmacotherapeutic strategies. **References-** [1] Cifani C. *et al.* A preclinical model of binge-eating elicited by yo–yo dieting and stressful exposure to food: effect of sibutramine, fluoxetine, topiramate and midazolam. Psychopharmacology 2009;204:113–25. [2] Micioni Di B MV *et al.* 2010 Appetite 54:663. [3] Violante IR *et al.* Cerebral activation by fasting induces lactate accumulation in the hypothalamus. Magn Reson Med. 2009; 62:279-83. [4] Engle MJ. *et al* Biochimica et Biophysica Acta 2001; 1511: 369-80.

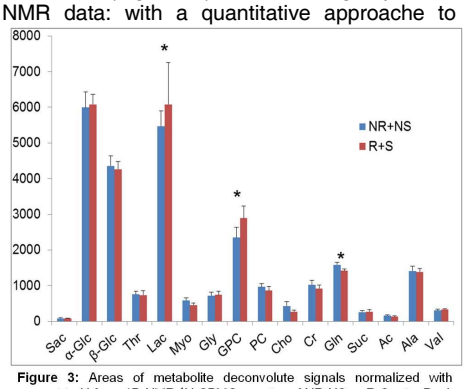

Figure 3: Areas of metabolite deconvolute signals normalized with respect to H form 1D NMR ^{1}H CPMG spectra of NR-NS or R-S rats .Peak areas were determined using Mnova software. Data were reported as means ± SEM. * $p < 0.05$ pairwise post-hoc Students's T-test.