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► **To cite this version:**

Mathilde Guerville, M Kristina Hamilton, Charlotte C Ronveaux, Sandrine Ellero-Simatos, Helen E Raybould, et al.. Chronic refined low-fat diet consumption reduces cholecystokinin satiation in rats. *European Journal of Nutrition*, 2019, 58 (6), pp.2497-2510. 10.1007/s00394-018-1802-2. hal-01862581

**HAL Id: hal-01862581**

**<https://univ-rennes.hal.science/hal-01862581v1>**

Submitted on 14 Sep 2018

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1 **Chronic refined low fat diet consumption reduces cholecystokinin satiation in rats.**

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9

10 **SHORT TITLE:** Refined diet reduces CCK satiation in rats

11

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19

**Abstract**

21 Purpose: Reduced ability of cholecystinin (CCK) to induce satiation contributes to  
22 hyperphagia and weight gain in high fat / high sucrose (HF/HS) diet-induced obesity, and has  
23 been linked to altered gut microbiota. Rodent models of obesity use chow or low fat (LF) diets  
24 as control diets; the latter has been shown to alter gut microbiota and metabolome. We aimed  
25 to determine whether LF diet consumption impacts CCK satiation in rats and if so, whether this  
26 is prevented by addition of inulin to LF diet.

27 Methods: Rats (n=40) were fed for 8 weeks a chow diet (chow) or low fat (10%) or high fat /  
28 high sucrose (45 and 17%, respectively) refined diets with either 10% cellulose (LF and HF/HS)  
29 or 10% inulin (LF-I and HF/HS-I). Caecal metabolome was assessed by <sup>1</sup>H-NMR-based  
30 metabolomics. CCK satiation was evaluated by measuring the suppression of food intake after  
31 intraperitoneal CCK injection (1 or 3 µg/kg).

32 Results: LF diet consumption altered the caecal metabolome, reduced caecal weight and  
33 increased IAP activity, compared to chow. CCK-induced inhibition of food intake was abolished  
34 in LF diet fed rats compared to chow-fed rats, while HF/HS diet-fed rats responded only to the  
35 highest CCK dose. Inulin substitution ameliorated caecal atrophy, reduced IAP activity and  
36 modulated caecal metabolome, but did not improve CCK-induced satiety in either LF or HF/HS  
37 fed rats.

38 Conclusions: CCK signaling is impaired by LF diet consumption, highlighting that caution must  
39 be taken when using LF diet until a more suitable refined control diet is identified.

40

41 Keywords: obesity, gut-brain axis, metabolomics, vagal afferents, food intake

42

## 43 INTRODUCTION

44 The gastrointestinal tract releases more than 20 different hormones that contribute to the  
45 regulation of satiety and hunger. The most extensively studied satiating hormone is  
46 cholecystokinin (CCK), which is synthesized and secreted in the duodenum in response to the  
47 presence of fat and protein in the lumen [1, 2]. In rodents, CCK acts synergistically with leptin  
48 to enhance the activity of vagal afferent neurons (VAN), relaying information to the nucleus of  
49 the solitary tract (NTS) and then to the hypothalamus [3]. Integration of this signal leads to  
50 reduction of food intake and meal termination [2, 4]. Regulation of food intake by the CCK-  
51 leptin system is impaired in certain conditions such as diet-induced obesity (DIO) [5, 6]. Indeed  
52 DIO-rodents are insensitive to low doses of CCK [5]. Decreased sensitivity to satiating signals  
53 may ultimately lead to hyperphagia and weight gain enhancement. Thus, understanding the  
54 mechanisms leading to altered CCK sensitivity is of utmost importance in the context of  
55 obesity and overweight pandemic. Leptin-resistance of VAN has been described as the causal  
56 mechanism of this loss of sensitivity to CCK [5]. Yet the full mechanisms leading to VAN leptin-  
57 resistance and ultimately decreased satiation signals are still not fully described.

58  
59 Most obesity studies using DIO rodent models examined differences between a control group  
60 fed a chow diet and animals fed a refined high fat/high sucrose (HF/HS) diet that provides 4 to  
61 6 times more fat than the chow diet [7]. Chow diet is composed of a large array of unrefined  
62 plant and animal products. Conversely, HF/HS diet is processed with purified ingredients, each  
63 of them providing one main macronutrient or micronutrient. Many differences can be found  
64 between these diets: the nature and quantity of protein, fat, sugars and dietary fibers as well  
65 as the level of micronutrients [7, 8]. Hence, when comparing the effects of HF/HS diet to that  
66 of chow, the impact of increased dietary fat is confounded with the effects of other components  
67 that differ between the diets [7, 8]. Refined low fat (LF) diet has also been widely used in DIO  
68 studies as control diet. However, using various diets and animal models, Chassaing et al  
69 observed that switching from chow to refined diets, irrespective of dietary fat content or protein  
70 nature, leads to altered gut homeostasis with major atrophy of the large intestine [9]. Among

71 the most striking effects was the dramatic change in caecal metabolome in rats fed a refined  
72 LF diet compared to chow-fed ones as demonstrated by the use of <sup>1</sup>H-RMN [9]. This suggests  
73 that refined LF diet consumption by itself induces gut microbiota dysbiosis. This effect of  
74 refined LF diet upon gut microbiota and gut morphology was recently confirmed by Dalby et al.  
75 [10]. Interestingly, substituting inulin, a soluble fiber, for cellulose in refined LF diet improved  
76 caecal metabolomic profile and prevented intestinal atrophy [9].

77  
78 One consistent observation in the literature is that altered VAN-mediated gut-brain  
79 communication is associated with dysbiosis and altered gut homeostasis. Indeed, using  
80 antibiotics to manipulate HF/HS diet-induced change in gut microbiota composition in rats, a  
81 recent study demonstrated that gut microbiota dysbiosis induces withdrawal of VAN from the  
82 gut and the NTS and increases expression of inflammatory markers in VAN [11]. Microglia  
83 activation in the nodose ganglia is also associated with altered gut barrier function and  
84 intestinal inflammation in rats fed high sucrose diets [12]. Moreover, lipopolysaccharides  
85 (LPS), gut bacterial components that translocate to the blood in DIO [13], have been identified  
86 as potential triggers of VAN dysfunction *in vitro* [14] and *in vivo* [15]. Taken together these data  
87 suggest that altered host-microbiota interactions in obesity participates in the disturbances of  
88 VAN-mediated CCK signaling to the brain[16]. We therefore hypothesized that because of the  
89 major effects of refined LF diet upon gut microbiota and gut morphology recently described,  
90 LF diet consumption itself would alter CCK signaling compared to a chow diet, independently  
91 of dietary fat content. We also hypothesized that by preventing LF diet-induced dysbiosis and  
92 gut atrophy, addition of inulin to a LF diet would improve vagal signaling to the brain and  
93 constitute a proper control diet that could be used for further study. We therefore formulated 4  
94 refined diets differing in fat and sucrose content and nature of fiber: low-fat cellulose (LF), low-  
95 fat inulin (LF-I), high-fat/high sucrose cellulose (HF/HS) and high-fat/high sucrose inulin  
96 (HF/HS-I) and fed these diets to rats for 8 weeks. We evaluated gut microbiota metabolome  
97 and gut morphology as well as the ability of CCK to induce meal termination in rats fed these  
98 refined diets compared to that of a group of rats fed chow.

## 99 MATERIAL AND METHODS

100

### 101 Animals

102 Male Wistar rats (9-week old, 280-370g Harlan San Diego, n=40) were maintained and  
103 handled in accordance with protocols approved by the Institutional Animal Care and Use  
104 Committee (University of California, Davis, USA). All animals were housed individually at 22°C  
105 with a 12:12 hour light-dark in 435 x 290 x 150 mm polycarbonate rat cage. Body weight and  
106 food intake were measured three times a week. Food intake was measured by weighing the  
107 remaining food in the cage lid top after careful examination of the cage to collect crumbs.  
108 Rats were fed a chow diet (Chow; Purina Lab Diet 5001 rodent diet, 3.4 kcal/g, Table 1) for  
109 two weeks during acclimation to the animal facility. Rats were then split into 5 weight-matched  
110 groups and fed either chow, low fat (LF; Research diets D12450H, 3.7 kcal/g, 10% Cellulose,  
111 Table 2), LF-inulin (LF-I; Research diets, 10% Inulin, 3.8 kcal/g, Table 2), high fat (HF/HS;  
112 Research diets D12451, 4.5 kcal/g, 10% Cellulose, Table 2) or HF/HS-inulin (HF/HS-I;  
113 Research diets, 4.7 kcal/g, 10% Inulin, Table 2) diets for 8 weeks (n=8 per group). Energy  
114 intake and feed conversion ratio (kcal ingested / weight gain) were calculated according to the  
115 nutritional information given by the supplier. Inulin was purchased from Beneo (Orafti® GR;  
116 Belgium) and supplemented into the LF and HF/HS diets by Research Diets. Inulin was  
117 extracted from chicory root and had an average chain length >10.

118

### 119 CCK sensitivity assessment

120 After 6 weeks on respective diets, rat sensitivity to the satiating effect of CCK was tested.  
121 Experiments were performed at the onset of the dark phase. Rats were fasted on wire-bottom  
122 cages for 12h during the light phase. At the onset of the dark phase, CCK (octapeptide,  
123 sulfated, Bachem, Torrance, CA, 1 µg or 3 µg/kg; i.p.) or saline (400 µL; i.p.) were administered.  
124 Food was placed in the cage and food intake recorded after 20 and 60 minutes. The dose of  
125 CCK was chosen based on previous work showing decreased sensitivity to CCK i.e. no  
126 satiating effect of the lowest CCK dose but intact effect of the highest dose in DIO (defined as

127 rats with significantly greater adiposity index than chow-fed rats) rats compared to chow-fed  
128 rats [14]. All rats received vehicle and CCK doses randomly, with a minimum of 48 hours  
129 between each fast.

130

### 131 **Tissue collection**

132 After 8 weeks on respective diets, rats were fasted overnight then injected leptin (80 µg/kg i.p.,  
133 n=4 per group) or saline (400µL, n=4 per group). They were euthanized 2 hrs later by cardiac  
134 puncture under deep anesthesia induced by isoflurane. Plasma was obtained by centrifugation  
135 (4000rpm, 10 min, 4°C) and stored at -80°C. Fat pads were dissected (mesenteric fat  
136 corresponding to the whole mesentery, epididymal fat located around the epididymes and  
137 retroperitoneal fat around the kidneys) and weighed. Adiposity was calculated as the sum of  
138 fat pad weights / body weight \* 100. Retroperitoneal fat sample (1 cm<sup>3</sup>) was fixed in 4%  
139 buffered paraformaldehyde for 24hrs then processed for paraffin embedding. Caecum and  
140 luminal contents were snap frozen in liquid nitrogen and stored at -80°C. Nodose ganglia were  
141 dissected and immediately snap frozen in liquid nitrogen and stored at -80°C.

142

### 143 **Intestinal alkaline phosphatase activity**

144 After homogenization of caecal samples, the activity of alkaline phosphatase was assayed in  
145 caecal homogenates with commercial kits according the manufacturer's instructions  
146 (Sensolyte, Anaspec, San Jose, CA, USA).

147

### 148 **Plasma LPS-binding protein**

149 Lipopolysaccharide-binding protein (LBP) levels were measured in serum samples via ELISA  
150 kit according to manufacturer's recommendations (Biometec, Greifswald, Germany).

151

### 152 **Histology of adipose tissue**

153 Retroperitoneal fat samples of 4 rats per dietary group were cut (10µm). Sections were stained  
154 with hematoxylin and eosin and images were taken at 100x magnification using the MetaMorph

155 Basic v. 7.7.0. image-analyzer software on an Olympus BX61 microscope. The area of  
156 adipocytes was measured with Image J 1.42p digital imaging processing software. Each image  
157 was converted into a binary format, and the area of adipocytes for each sample was analyzed  
158 in six random microscopic fields. All measurements were done blinded for diet group.

159

#### 160 **Western blot PTP1b**

161 Proteins from nodose ganglia were extracted with Tris-base EDTA buffer (1%Triton, 1%  
162 protease inhibitor, 1% Phosphatase inhibitor and 3%PMSF). Samples (5µg of protein) were  
163 loaded into precast 10% BisTris gels (Invitrogen NuPage) and migrated for 50 minutes at 200V.  
164 The proteins were transferred on a PDVF membrane (Biorad #162-0174 7.0cm x 8.5cm) for 1  
165 h at 30V. Membrane was blocked using 10% BSA in PBS for 1 h at room temperature. Anti-  
166 protein tyrosine phosphatase 1b (PTP1b) (Rabbit, Abcam, ab189179) was diluted at 1:500 and  
167 anti-GADPH (Rabbit, Cell Signalling, 14C10) was used as a loading control. Primary antibodies  
168 were applied on the membrane and developed on separate but consecutive days. Antibodies  
169 were incubated for 1 h at room temperature and then overnight at 4°C. The membrane was  
170 imaged using ECL substrate (Thermo Scientific) and with ChemiDoc XRS Imager (BioRad,  
171 Hercules, CA). The membrane was analyzed by Image Lab version 5.0 software (Hercules,  
172 CA)

173

#### 174 **<sup>1</sup>H NMR metabolomics**

175 Caecal extracts for NMR spectroscopy were prepared by mixing 50 mg of the caecal content  
176 with 500 µL of phosphate buffer (0.2 M, pH 7.4) containing 90% D<sub>2</sub>O, 1% (w/v) of sodium 3-  
177 (trimethylsilyl) propionate (TSP), and 0.3 mM NaN<sub>3</sub>. After vortexing, each sample was  
178 subjected to a freeze–thaw cycle in liquid nitrogen and subsequently homogenized with a  
179 tissue lyser (QIAGEN, Hilden, Germany) at 20 Hz for 40 s followed by centrifugation at  
180 10000xg for 10 min at 4°C. The supernatants were collected, and the remaining pellet was  
181 extracted once more as described above. Supernatants obtained from the two extractions



182 were combined and centrifuged at 10000×g for 10 min at 4°C. A total of 600 µL of supernatant  
183 was transferred into an NMR tube (outer diameter, 5 mm) pending NMR analysis. All <sup>1</sup>H-NMR  
184 spectra were obtained on a Bruker DRX-600-Avance NMR spectrometer (Bruker,  
185 Wissembourg, France) on the AXIOM metabolomics platform (MetaToul, Toulouse, France)  
186 operating at 600.13 MHz for <sup>1</sup>H resonance frequency using an inverse detection 5-mm <sup>1</sup>H-<sup>13</sup>C-  
187 <sup>15</sup>N cryoprobe attached to a cryoplatfrom (the preamplifier cooling unit). The <sup>1</sup>H-NMR spectra  
188 were acquired at 300K using the Carr-Purcell-Meiboom-Gill spin-echo pulse sequence with  
189 pre-saturation and a total spin-echo delay (2πτ) of 100 ms. A total of 128 transients were  
190 collected into 64,000 data points using a spectral width of 12 ppm, a relaxation delay of 2.5 s,  
191 and an acquisition time of 2.28 s. Data were analyzed by applying an exponential window  
192 function with a 0.3-Hz line broadening prior to Fourier transformation. The resultant spectra  
193 were phased, baseline corrected, and calibrated to TSP (δ 0.00) manually using Mnova NMR  
194 (v9.0, Mestrelab Research). The spectra were subsequently imported into MatLab (R2014a,  
195 MathsWorks, Inc.) All data were analyzed using full-resolution spectra. The region containing  
196 the water resonance (δ 4.6–5.2ppm) was removed, and the spectra were normalized to the  
197 probabilistic quotient [17] and aligned using a previously published function [18].

198  
199 Data were mean-centered prior to analysis using orthogonal projection on latent structure-  
200 discriminant analysis (O-PLS-DA). <sup>1</sup>H-NMR data were used as independent variables (X  
201 matrix) and regressed against a dummy matrix (Y matrix) indicating the class of samples. O-  
202 PLS-derived model was evaluated for goodness of prediction (Q<sup>2</sup>Y value) using 8-fold cross-  
203 validation. The reliability of each model was established using a permutation test of the Y  
204 vector (1000 permutations) in order to determine a p-value for each Q<sup>2</sup>Y. To identify  
205 metabolites responsible for discrimination between the dietary groups, the O-PLS-DA  
206 correlation coefficients (r<sup>2</sup>) were calculated for each variable and back-scaled into a spectral  
207 domain, so that the shape of NMR spectra and the sign of the coefficients were preserved[19].  
208 The weights of the variables were color-coded, according to the square of the O-PLS-DA  
209 correlation coefficients. Correlation coefficients extracted from significant models were filtered

210 so that only significant correlations above the threshold defined by Pearson's critical correlation  
211 coefficient ( $P < 0.05$ ;  $|r| > 0.71$ ) were considered significant. For illustration purposes, the area  
212 under the curve of several signals of interest was integrated and statistical significance was  
213 tested using univariate tests.

214

### 215 **Statistical analysis**

216 Statistical analysis was performed on GraphPad Prism software (v5, San Diego, CA USA) and  
217 data were expressed as means  $\pm$  SEM. Significance was determined by one-way ANOVA  
218 for cumulative energy intake, feed conversion ratio, liver weight, adiposity, caecal weight and  
219 IAP activity. For the CCK sensitivity assessment, the significance was determined by two-way  
220 ANOVA testing the effect of the CCK dose, diet and of the interaction between these two  
221 factors. For body weight, data were analyzed using two-way ANOVA testing the effect of time,  
222 diet and the interaction between those two factors. Finally, frequency of adipocyte size was  
223 analyzed using two-way ANOVA testing the diet, adipocyte size class and the interaction  
224 between factors.

## 225 RESULTS

226

### 227 **LF and HF/HS diets had different effects on rat phenotype which were modulated by** 228 **inulin**

229 Rats fed HF/HS diet gained more weight compared to both chow and LF diet-fed rats during  
230 the 8-week feeding period (Fig. 1A), resulting in a greater body weight after 6, 7 and 8 weeks  
231 on the HF/HS diet (Fig. 1B). Substitution of inulin for cellulose in the HF/HS diet did not change  
232 rat weight gain after 8 weeks of diet consumption, with HF/HS-I diet-fed rats exhibiting body  
233 weight gain similar to that of HF/HS diet-fed rats and greater than that of chow, LF- and LF-I  
234 diet-fed rats (Fig. 1A and B).

235 Average daily energy intake of HF/HS diet-fed rats was greater at week 1 compared to that of  
236 the other dietary groups (Fig. 1C). Average daily energy intake was stable after 2 weeks and  
237 not different from that of the other groups. This resulted in a cumulative energy intake similar  
238 in HF/HS-diet and chow-fed rats (Fig. 1D). Feed conversion ratio (kcal/weight gain) was lower  
239 in HF/HS-diet fed rats compared to chow-fed rats (Fig. 1E). Average daily energy intake was  
240 not significantly different from that of chow-fed rats and stable after 2 weeks on refined diets  
241 (Fig. 1D). However, when expressed as cumulative energy intake, LF diet-fed rats exhibited a  
242 lower cumulative energy intake compared to chow and HF/HS diet-fed rats (Fig. 1D). They had  
243 a similar feed conversion ratio to that of HF/HS diet-fed rats (Fig. 1E). Inulin did not change  
244 caloric intake in LF-I diet-fed rats which exhibited lower cumulative food intake compared to  
245 chow-fed rats (Fig. 1C and D). A slight reduction in cumulative caloric intake was observed in  
246 HF/HS-I diet-fed rats compared to HF/HS-diet fed ones ( $P=0.06$ , Fig. 1D).

247 Plasma LBP was not different between chow- and refined diet-fed rats (Fig. 1F). However, LF-  
248 I diet-fed rats exhibited a significantly greater level of LBP compared to LF diet-fed ones (Fig.  
249 1F).

250 Relative to chow-fed rats, HF/HS diet-fed rats exhibited marked adiposity with an increased  
251 adiposity index (Fig. 1G) characterized by elevated retroperitoneal, mesenteric and epididymal  
252 fat pad mass (data not shown). This increased adiposity was attributable at least in part to

253 adipocyte hypertrophy as demonstrated by the significant increase in the frequency of large  
254 adipocytes (size comprised between 8 and 10 000  $\mu\text{m}^2$ ) and decrease in smaller ones (size  
255 comprised between 4 and 6 000  $\mu\text{m}^2$ ) in HF diet-fed compared to chow-fed rats (Fig. 1H).  
256 Adiposity of LF diet-fed rats was not significantly different from that of chow or HF/HS diet-fed  
257 rats (Fig. 1G). However, adipocyte size distribution was slightly altered by LF diet consumption  
258 since the frequency of intermediate size adipocytes (size comprised between 6 and 8 000  $\mu\text{m}^2$ )  
259 was significantly increased and that of smaller adipocytes (size comprised between 4 and  
260 6 000  $\mu\text{m}^2$ ) decreased in LF diet- compared to chow-fed rats (Fig. 1H). Inulin did not change  
261 adiposity which was still greater in HF/HS-I-diet fed rats than chow-fed ones (Fig. 1G).  
262 Adipocyte size distribution in HF/HS-I diet-fed rats was not significantly different from that of  
263 HF/HS diet-fed ones but also not different from that of chow-fed ones, suggesting slight  
264 improvement of adipocyte hypertrophy. This improvement in adipocyte size distribution was  
265 visible in LF-I diet-fed rats which exhibited significant decrease in the frequency of adipocytes  
266 with a size between 6 and 8 000  $\mu\text{m}^2$  and significant increase in smaller adipocytes (4 -  
267 6 000 $\mu\text{m}^2$ ) compared to LF-diet fed rats (Fig. 1H).

268 Liver weight was not impacted by any of the diets (data not shown).

269

### 270 **LF and HF/HS diets had similar impacts on caecal metabolome, morphology and** 271 **defense mechanisms, which were partially prevented by inulin**

272 We investigated the diet-induced alterations in gut microbiota metabolome using  $^1\text{H-NMR}$   
273 based metabolomics in caecal extracts. Typical spectrum with identified metabolites is  
274 displayed in Suppl. Fig. 1.

275 The caecal metabolomic profile of chow-fed rats was significantly different from those of LF-  
276 or HF/HS diet-fed rats as demonstrated by the OPLS-DA scores showing clear clustering of  
277 chow-fed vs LF- or HF/HS diet-fed rats (Fig. 2A and Table 3). The caecal metabolomes of LF-  
278 and HF/HS diet-fed rats were not significantly different from each other, as reflected by the  
279 non-significant OPLS parameters (Table 3) and the non-separation of the 2 groups on the  
280 OPLS scores (Fig. 2B). OPLS models discriminating the caecal metabolic profiles from chow-

281 vs. LF diet-fed rats and chow- vs. HF/HS diet-fed rats were used to identify metabolites  
282 responsible for the discrimination between metabolomes (Fig. 2C and D).

283 More specifically, we observed that consumption of LF or HF/HS diets had a minimal impact  
284 on the caecal levels of bile acids and short chain fatty acids (Table 4). However, refined diets  
285 consumption increased the caecal levels of many amino acids such as isoleucine, lysine, valine  
286 in LF diet-fed rats and isoleucine in HF/HS-diet fed rats (Table 4). Similar trends for alanine,  
287 aspartate and phenylalanine were also observed. Conversely, the levels of the valine  
288 metabolite,  $\alpha$ -keto-isovalerate, was decreased and that of 5-aminvalerate increased with LF  
289 diet consumption (Table 4). A tendency for decreased levels of the aromatic amino acid  
290 metabolite, 4-hydroxyphenylacetate (4-HPPA) in the caecum of LF diet-fed rats was observed.  
291 Increased level of the ketone body  $\beta$ -hydroxybutyrate and a tendency for decreased level of  
292  $\alpha$ -keto-glutarate were observed in the caecum of HF/HS diet-fed rats, with similar trends in LF  
293 diet-fed rats (Table 4). The level of two unknown metabolites and of hypoxanthine was  
294 decreased in rats fed the LF or HF/HS-diets (Table 4).

295 Addition of inulin to the refined diets impacted the caecal metabolome of LF diet-fed rats but  
296 not of HF/HS-diet fed ones. The caecal metabolome of LF-I fed-rats was different from that of  
297 both chow- and LF diet-fed rats as demonstrated by the clear separation of the 3 groups on  
298 the O-PLSDA (Fig. 2E and Table 3) while that of HF/HS-I and HF/HS diet-fed rats were not  
299 different (Fig. 2F and Table 3). Because of the strong difference between caecal metabolomic  
300 profiles of chow- vs. refined diet-fed rats, we also analyzed metabolomic data after omitting  
301 the chow-fed rat group. The OPLS model discriminating the 4 refined diets confirmed the  
302 significant impact of inulin substitution on caecal metabolome, especially in the LF diet (Fig.  
303 2G and Table 3). Metabolites responsible for the difference between LF- and LF-I diet-fed rat  
304 caecal metabolomic profiles are displayed on Fig. 2F.

305 Specifically, inulin substitution increased the caecal content levels of butyrate (significant for  
306 LF-I diet, Table 4). LF-I and HF/HS-I diet-fed rats had reduced caecal levels of isoleucine and  
307 valine and increased levels of choline and hypoxanthine compared to LF and HF/HS diet-fed  
308 rats, with the greatest effects seen in LF-I diet-fed rats. Inulin substitution tended to decrease

309 caecal levels of the ketone body  $\beta$ -hydroxybutyrate, to values closer to that of chow fed-rats  
310 (Table 4). Inulin had no effect on the variations in lysine,  $\alpha$ -ketoisovalerate, trimethylamine and  
311 the two unknown compounds induced by LF- and HF/HS-diet consumption (Table 4).  
312 Surprisingly, inulin substitution enhanced the increase in aspartate and phenylalanine and the  
313 decrease in 4-HPPA caecal contents already observed with LF- and HF/HS-diet consumption.  
314 Moreover, it increased the level of alanine and tyrosine in the caecal content of LF-I and  
315 HF/HS-I diet-fed rats. Finally, glutamate level was increased and 5-aminovalerate level  
316 decreased in the caecal content of LF-I diet-fed rats (Table 4).

317  
318 Consumption of LF and HF/HS diets induced gut atrophy with decreased caecal tissue weight  
319 in LF- and HF/HS diet-fed compared to chow-fed rats (Fig. 2I). Caecal atrophy was prevented  
320 by addition of inulin in the diets (Fig. 2I). We also evaluated the caecal activity of intestinal  
321 alkaline phosphatase (IAP) a key brush-border enzyme in the defense mechanisms of the  
322 mucosa which has been shown to be strongly induced in DIO and plays an essential role in  
323 intestinal homeostasis and health through interactions with the resident microbiota, diet and  
324 the gut[20]. Relative to chow-fed rats, LF diet-fed rats exhibited a 15-fold increase in caecal  
325 IAP activity (Fig. 2J). Caecal IAP activity was increased 7-fold in HF/HS diet-fed rats but this  
326 did not reach significance (Fig. 2J). The increase in IAP activity in the caecum induced by LF-  
327 diet consumption was prevented by addition of inulin (Fig. 2J)

328  
329 **LF and HF/HS diet-fed rats exhibited reduction in sensitivity to CCK and VAN leptin**  
330 **resistance, which were not improved by inulin**

331 Chow-fed rats displayed decreased calorie intake during the first 20 min following  
332 administration of both doses of CCK (Fig. 3A and B), with no difference in calorie intake  
333 between the two doses (-41 and -56% for CCK 1  $\mu$ g/kg and 3  $\mu$ g/kg, respectively,  $P < 0.01$   
334 compared to saline). After 20 min, CCK injection had no further effect on caloric intake in chow-  
335 fed rats (Fig. 3B). As expected, HF/HS diet-fed rats displayed decreased calorie intake during  
336 the first 20 min following the highest dose of CCK (-56%,  $P < 0.05$  compared to saline) but were

337 insensitive to the low dose (Fig. 3A and B). Surprisingly, LF diet-fed rats ate less calories than  
338 chow-fed rats under the saline condition (-41%,  $P=0.01$  compared to chow-fed rats under  
339 saline). LF diet-fed rats administered either dose of CCK did not significantly decrease calorie  
340 intake during the first 20 minutes. This absence of effect of CCK on caloric intake in HF/HS-  
341 fed rats and LF-fed rats was not due to a delayed action of CCK since no effect of CCK was  
342 observed on caloric intake between 20 and 60 minutes in these animals (Fig. 3C and D).

343 Addition of inulin to the LF or HF/HS- diets had no significant effect on CCK-induced inhibition  
344 of food intake during the first 20 minutes (Fig. 3A and B). LF-I diet fed rats did not respond to  
345 either dose of CCK. Moreover, administration of CCK  $3\mu\text{g}/\text{kg}$  significantly inhibited 20-min  
346 caloric intake by 58% ( $P<0.01$  compared to saline) in HF/HS-I fed rats, but there was no  
347 response to the lower dose of CCK. (Fig. 3A and B). This difference in response to CCK  
348 compared to chow-fed animals was not due to a delayed effect of CCK since the 20-60 min  
349 caloric intake was not affected by CCK injection in LF-I or HF/HS-I diet fed rats (Fig. 3C and  
350 D).

351  
352 We investigated whether rats fed the refined diets were sensitive to leptin by injecting rats with  
353 leptin or saline 2hrs before euthanasia. The level of PTP1b in the nodose ganglia evaluated  
354 by western blot was used as a marker of leptin downstream signaling. Due to technical  
355 problems, the nodose ganglia of only 2 chow-fed rats injected with leptin were analyzed, thus  
356 the following observations will be interesting to validate in future studies. The level of PTP1b  
357 after leptin injection increased compared to saline in chow-fed rats (Table 5). Conversely, leptin  
358 injection did not change PTP1b level in the nodose ganglia of LF-, LF-I and HF/HS-diet fed  
359 rats (Table 5) while it decreased PTP1b level in the nodose ganglia of HF/HS-I diet-fed rats  
360 ( $P=0.01$ , Table 5). These preliminary data suggest that rats fed the refined diets have altered  
361 leptin signaling in VAN compared to chow-fed ones.

362

363 **Decrease in sensitivity to CCK correlated with adiposity parameters and plasma LBP**

364 The most discriminant parameter between rats fed chow or the refined diets was the response  
365 to low dose CCK, which was blunted in refined diet-fed rats. Yet, a large variability in this  
366 parameter was observed between rats, even within a dietary group. We decided to take  
367 advantage of this variability to investigate if this parameter correlated with any phenotypic or  
368 metabolomic data (coefficient correlation in Suppl. Table 1).

369 The amount of calorie ingested during 20 min after CCK 1 $\mu$ g/kg injection correlated  
370 significantly and positively with several indexes of adiposity: adiposity index (Fig. 4A),  
371 mesenteric fat relative weight, retroperitoneal fat relative weight (Fig. 4B), epididymal fat  
372 relative weight (Fig. 4C), retroperitoneal fat adipocyte average size and frequency of large  
373 adipocytes in retroperitoneal fat (Fig. 4D). It also correlated positively with the caecal content  
374 levels of alanine and ethanol and negatively with that of 4-HPPA (Suppl. Table 1).

375 Because caloric intake of LF- and LF-I diet-fed rats was lower than that of the other groups  
376 even under saline, we also tested the change in calorie intake after CCK 1 $\mu$ g/kg injection  
377 compared to saline injection as an index of CCK sensitivity. Similarly, we found that this  
378 parameter correlated with adiposity index and the retroperitoneal fat pad relative weight (Fig.  
379 4E). The decrease in calorie intake correlated significantly with plasma LBP (Fig. 4F). It also  
380 correlated significantly with the caecal content level of aspartate, one bile acid and one  
381 unknown compound (Suppl. Table 1).

382



**383 DISCUSSION**

384

385 Recent data have shown gut microbiota dysbiosis in rodents fed a refined LF diet and there is  
386 increasing evidence showing a link between gut dysbiosis and alteration in the CCK-leptin-  
387 VAN signaling pathway controlling satiation. We hypothesized that LF diets impair CCK-  
388 induced inhibition of food intake and that inulin could prevent this effect. We confirmed that LF  
389 diet consumption for 8 weeks impacted caecal metabolome compared to chow, suggesting  
390 altered microbiota function similar to that induced by a HF/HS diet. We also showed that LF  
391 diet consumption resulted in the loss of CCK satiation similar to that observed with HF/HS diet.  
392 Substituting inulin, a soluble fiber, for cellulose, an insoluble fiber, in refined diets minimally  
393 impacted the phenotype but prevented caecal atrophy and alteration of gut defense, and  
394 modulated the caecal metabolome. Interestingly, addition of inulin to either diet had no effect  
395 on CCK-induced inhibition of food intake. Taken together, these data suggest that although  
396 addition of a soluble fiber to synthetic diets may improve some aspects of gut microbial  
397 dysbiosis and host gut function, soluble fiber does not restore gut-brain signaling, specifically  
398 activation of vagal afferents by CCK.

399

400 Recent data suggest that the type of control diet (LF or chow) used in DIO studies can  
401 profoundly change the conclusion drawn from the studies since consumption of LF refined  
402 diets has been shown to alter host physiology and metabolism [21]. Our study is the first to  
403 investigate the effects of refined diet feeding on CCK induced inhibition of food intake. Low  
404 dose CCK injection did not induce a reduction in food intake in rats fed the refined diets, either  
405 LF or HF/HS, suggesting that similar to HF/HS-diet fed rats, LF-diet fed rats had reduced  
406 sensitivity to CCK compared to chow-fed rats. One caveat to this interpretation is that food  
407 intake was reduced significantly in LF diet-fed compared to chow-fed rats even after vehicle  
408 administration. Although there was no further inhibition of food intake with administration of  
409 CCK, it is possible that food intake had reached a “floor effect” where it was not possible to  
410 further reduce food intake in fasted rats. The reason for the decreased food intake under saline

411 as well as the decreased cumulative energy intake over the 8-week feeding period in LF-diet  
412 fed rats is unknown. Feed conversion ratio, i.e. the calorie needed to gain weight was reduced  
413 in LF- diet fed rats compared to chow-fed ones. This suggests that LF diet-fed rats extracted  
414 enough energy and nutrients from the LF diet to sustain growth rate similar to that of chow.  
415 The presence of purified ingredients in the refined diet probably accounts for this lower feed  
416 conversion ratio. Similar results, i.e. decreased energy intake and lower feed conversion ratio  
417 in LF-diet fed rats compared to chow-fed ones were observed by Sen et al. [12].

418  
419 CCK sensitivity, expressed as calories ingested after injection of the low dose of CCK or as  
420 the decrease in calorie intake after low dose of CCK injection, compared to saline injection,  
421 correlated significantly to adiposity parameters. Despite similar growth rate, LF diet-fed rats  
422 exhibited a trend for increase in adiposity index compared to chow-fed rats. The shape of their  
423 adipocyte size distribution curve was shifted towards larger adipocytes compared to chow-fed  
424 rats, suggesting accumulation of lipids in adipose tissue. Small, yet significant, increase in fat  
425 pad weight has also been observed in mice or rats fed a refined LF diet compared to chow in  
426 previous studies [9, 12, 21, 22]. Several studies reported muscle insulin resistance of mice or  
427 rats fed a LF diet even early after the start of LF diet consumption [21, 22], leading to pancreas  
428 compensation and high level of circulating insulin. In these studies though, LF diets contained  
429 high levels of sucrose which could explain these metabolic perturbations. Our LF diet did not  
430 contain sucrose. Thus the slight increase in adiposity in LF diet-fed rats which correlate  
431 significantly with CCK sensitivity, would need further investigation.

432  
433 We hypothesized that the lack of activation of VAN by CCK in refined diet-fed rats was a result  
434 of leptin resistance. Several reports indicate that leptin resistance can be induced in relatively  
435 short periods at normal body fat and leptin levels [23]. Our attempt to investigate leptin  
436 signaling *via* the downstream mediator of leptin PTP1b in the nodose ganglia raised preliminary  
437 but encouraging results suggesting impaired leptin signaling. However, this needs to be  
438 confirmed. Another hypothesis is that the sucrose level in the diets could explain the lower

439 sensitivity to CCK in our refined-diet fed rats as demonstrated by Sen et al. [12]. However, our  
440 LF-diet did not contain sucrose. We therefore hypothesized that dysbiosis-induced  
441 translocation of LPS triggers impairment of leptin signaling and sensitivity to CCK in VAN as  
442 already demonstrated [14, 15]. *In vitro*, infusion of LPS on VAN primary culture increased  
443 suppressor of cytokine signaling 3 (SOCS3) expression, a marker of leptin resistance, in a  
444 dose-dependent manner [14]. Similarly, rats implanted with osmotic mini-pumps that  
445 chronically deliver LPS for 6 weeks exhibited increased SOCS3 expression in VAN, associated  
446 to decreased phosphorylation of signal transducers and activators of transcription 3, another  
447 marker of leptin signaling disruption [15]. HF/HS chronic consumption has been shown to  
448 induce dysbiosis and alter the intestinal and hepatic mechanisms involved in protection against  
449 LPS entry into the organisms, resulting in greater plasma LPS in HF/HS diet fed rats [24]. The  
450 impact of LF diet consumption on intestinal barrier function has not been evaluated so far. In  
451 the current study, we evaluated plasma LBP level and IAP activity, a major enzyme involved  
452 in defense mechanisms, especially against LPS [13] and can be induced by LPS itself [25].  
453 The increased IAP activity observed in LF diet-fed compared to chow-fed rats could be a host  
454 adaptive response to altered microbiota composition and increased abundance of LPS-bearing  
455 bacteria as observed in DIO rats[24]. LBP is an acute-phase protein synthesized by  
456 hepatocytes in response to LPS and released into the bloodstream. As such plasma LBP is a  
457 useful marker of exposition to LPS [13]. Plasma LBP was not significantly different between  
458 chow- and refined-diet fed rats but was greater in LF-I diet-fed rats compared to LF diet-fed  
459 ones. Moreover, a significant positive correlation between calorie intake under CCK 1 $\mu$ g/kg  
460 and plasma LBP level was observed. Thus increased translocation of LPS in refined diet-fed  
461 rats might have disturbed signaling in VAN.

462

463 We show a significant difference in the caecal metabolomic profiles of C- vs. refined diet-fed  
464 rats, including LF-diet fed rats. Metabolic profiling has emerged as a valuable tool to evaluate  
465 modulation of gut microbiota metabolism in response to physiological or pathological changes.  
466 It can also serve as a fingerprint of biochemical perturbations unique to the nature or

467 mechanism of a particular biological process [26]. By analyzing the caecal metabolome using  
468 <sup>1</sup>H-NMR we were able to evaluate the consequences of LF-diet consumption on the levels of  
469 molecules directly in contact with the host. LF diet-fed rats exhibited lower caecal levels of  
470 several nucleic acids (hypoxanthine and a tendency for uracil). Similar decrease in uracil and  
471 hypoxanthine levels has been reported in the feces of DSS-induced colitic mice compared to  
472 healthy ones [27]. LF diet-fed rats also exhibited increased caecal levels of amino acids  
473 (isoleucine, lysine, phenylalanine, valine) with a concomitant decrease in the level of  $\alpha$ -keto-  
474 isovalerate and the same tendency for 4-HPPA, two microbial products of amino acid  
475 metabolism. The level of short chain fatty acids (SCFA) acetate and propionate also tended to  
476 be lower in the caecum of LF diet- compared to chow-fed rats, although not significant. Similar  
477 metabolomics profiles with increased levels of amino acids and decreased levels of short chain  
478 fatty acids have been reported in the feces of ulcerative colitis patients [28]. The similarity  
479 between the intestinal metabolome of our LF diet-fed rats and of animal models and humans  
480 with an inflamed gut suggests intestinal inflammation after LF diet consumption. Inflammation  
481 occurring with a LF diet has also been observed in the adipose tissue of mice which exhibited  
482 greater MCP-1 mRNA levels compared to chow-fed mice [21]. This suggests that refined LF  
483 diet can promote inflammation, either intestinal or systemic. It is possible that some of the  
484 effects of refined diets might be dependent on the lack of soluble fiber [9].

485

486 We chose to investigate how the substitution of inulin for cellulose in our refined diets affected  
487 CCK signaling given that inulin has been previously shown to prevent gut microbiota alteration  
488 and gut atrophy, and these parameters have been linked to CCK signaling. Dietary inulin did  
489 not modify weight gain and adiposity in HF/HS-I diet -fed rats. Only a slight improvement in the  
490 adipocyte size distribution was observed. Many publications have shown the beneficial effects  
491 of adding soluble fiber to purified diets to improve health in rodent models. In the context of  
492 purified ingredient diets, the addition of soluble fiber like inulin, fructo-oligosaccharides, and  
493 pectin improves gut morphology and reduce body weight and adiposity relative to insoluble  
494 cellulose[29–36]. However, some studies also reported poor effect of inulin or inulin-

495 oligofructose mix on weight gain and adiposity [9, 37, 38]. Specifically, Zou et al. showed that  
496 inulin added to a HF/HS diet did not decrease weight gain compared to the same amount of  
497 cellulose added to a HF/HS diet [36]. Moreover, recent data demonstrated worsening of  
498 intestinal inflammation with inulin or fructo-oligosaccharides in refined diet-fed mice colitis  
499 models [39, 40], suggesting that the effect of fermentable fiber in refined diet is not as clear.  
500 Difference in dose, type of inulin, time of dietary intervention but also the type/cause of  
501 inflammatory condition probably explain the discrepancies between studies.

502  
503 Despite the absence of effect of inulin on adiposity in our model, dietary inulin clearly prevented  
504 caecal atrophy and the increased in IAP activity. It also significantly changed the caecal  
505 metabolomic profiles by preventing the changes in the caecal level of several metabolites  
506 (isoleucine, valine,  $\alpha$ -keto-isovalerate, hypoxanthine, uracil) and by increasing butyrate caecal  
507 level in LF-I diet-fed rats. Yet, inulin also increased the caecal level of several other amino  
508 acids, especially in LF-I diet-fed animals (alanine, aspartate, tyrosine and glutamate) and  
509 enhanced some changes already observed with LF and HF/HS diets, further increasing the  
510 concentration of phenylalanine and decreasing that of 4-HPPA in the caecal lumen of rats.  
511 Inulin supplementation has previously been associated with increased mucin secretion in the  
512 caecal lumen in rats fed refined diets [41] and increased abundance of proteolytic bacteria  
513 including *Enterococcus* and bacteria belonging to the genus *Clostridium* [42]. Concomitant  
514 elevated mucus secretion and proteolytic activities could explain the elevated amino acid levels  
515 in the caecal lumen of inulin fed rats. Furthermore, fermentation of amino acids by bacteria is  
516 decreased at low pH compared to neutral pH [43]. Relative to cellulose, adding inulin to a diet  
517 decreases caecal pH [41, 44] and might therefore reduce amino acid bacterial utilization,  
518 consistent with the decreased level of amino acid metabolism end-products 4-HPPA and  $\alpha$ -  
519 keto-isovalerate. Besides modulating pH, inulin supplementation has also been associated  
520 with modulation of SCFA bacterial production despite conflicting results in the literature. In our  
521 model, dietary inulin only slightly changed SCFA levels in the caecum, with only a modest  
522 increase in butyrate level in LF-I diet-fed animals. In mice, one study reported that inulin

523 supplementation increased acetate production but did not modify butyrate and propionate  
524 caecal levels [9]. In humans, two studies showed decreased SCFA concentrations during inulin  
525 supplementation [45, 46]. Despite no effects of inulin on SCFA level in our model, inulin  
526 supplementation prevented caecal tissue atrophy as previously observed [9, 41, 44]. We  
527 speculate that inulin enhanced gut microbiota SCFA production and subsequent absorption of  
528 SCFA by the colonocytes, preventing their detection in the caecal metabolome of inulin-fed  
529 rats but resulting in colonocytes proliferation and caecal weight maintenance. Finally, although  
530 inulin added to refined diets improved caecal homeostasis, metabolomics data indicate that  
531 the gut microbiota of inulin-fed rats is still different from that of chow-fed rats, suggesting that  
532 host-microbiota interactions in these inulin-fed animals are probably still different from that of  
533 chow-fed rats.

534

535 In conclusion, our study provides evidence that refined LF diet consumption for 8 weeks  
536 reduced rat CCK sensitivity and profoundly impacted caecal metabolome which was similar to  
537 that of HF/HS-diet fed rats. Inulin prevented caecal atrophy and prevented some, but not all,  
538 of the metabolomic alterations induced by refined diet consumption. Overall, our data highlight  
539 the fact that even if chow is not the preferred control diet in terms of dietary constituents, it  
540 must be included as a proper control of microbiota-host homeostasis and normal rodent  
541 development defined by years of previous research. Caution must be taken when using LF  
542 diet which profoundly alters the equilibrium in the gut until a more suitable refined control diet  
543 is identified.

544

#### 545 **Acknowledgments**

546 The authors would like to thank Cécile Canlet from the French National Infrastructure of  
547 Metabolomics and Fluxomics (MetaboHUB-ANR-11-INBS-0010) for her help with the NMR  
548 facility and Dr. Olivier Cloarec from Korrigan Sciences Limited for providing the matlab  
549 functions for analysis of NMR data. They also want to thank Ricky Stephens for his help in  
550 animal experiment.

551 MG, MKH, CCR, HER and GB designed research, MG, MKH, CCR, SES conducted research,  
552 MG, SES analyzed data, MG, MKH, SES, HER and GB wrote the paper. GB had primarily  
553 responsibility for final content. All authors read and approved final version.  
554 The authors have no conflict of interest to declare.

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**Supporting information:**

**S1 Fig 1.** Assigned 600 MHz 1D NMR spectra of mouse caecal content. The 5 to 9 ppm region was vertically expanded 6 times compared to the 0 to 4.5 ppm region. Keys: 1: bile acids (mixed), 2: butyrate, 3: leucine, 4: isoleucine, 5: valine, 6: propionate, 7:  $\alpha$ -ketoisovalerate, 8: ethanol, 9:  $\beta$ -hydroxybutyrate, 10: lipids, 11: lactate, 12: alanine, 13: lysine, 14: acetate, 15: N-acetyl groups, 16: glutamate, 17: succinate, 18:  $\alpha$ -ketoglutarate, 19: aspartate, 20: choline, 21: taurine, 22:  $\beta$ -xylose, 23:  $\beta$ -galactose, 24:  $\beta$ -glucose, 25:  $\alpha$ -arabinose, 26:  $\alpha$ -xylose, 27:  $\alpha$ -glucose, 28:  $\alpha$ -galactose, 29: uracil, 30: tyrosine, 31: phenylalanine, 32: adenine, 33: hypoxanthine, 34: formate.

**S2 Table 1.** Correlation coefficients

## Figures legend

### Figure 1: Effect of diets on rat phenotype.

Percent body weight gain (A), weight gain (B), average daily energy intake (C), cumulative food intake (D), feed conversion ratio (E) over the 8-week period, plasma LPS-binding protein (F), adiposity index (G) and distribution of adipocyte size in retroperitoneal fat pads (H) after 8 week of Chow (black), LF (grey), LF-I (striped grey), HF/HS (white) and HF/HS-I (dotted white) -diet consumption.

Data are expressed as mean  $\pm$  SEM, (n=8 rats per group, except LBP n=5 to 8 and adipocyte size n=4). Means with different letters are significantly different ( $P < 0.05$ ). \*  $P < 0.05$  HF- vs. chow-diet, \$  $P < 0.05$  HF-I vs. chow-diet.

### Figure 2: Effect of diets on caecal metabolome, morphology and IAP activity.

O-PLS-DA cross-validated score plots showing the discrimination between  $^1\text{H-NMR}$  spectra of caecal contents from rats fed Chow vs refined LF vs HF/HS diets (A), HF/HS vs LF (B), Chow vs refined diet LF vs LF-I diets (E), Chow vs HF/HS vs HF/HS-I diets (F) and LF vs LF-I vs HF/HS vs HF/HS-I diets (G).

O-PLS-DA correlation loading plots relative to the discrimination between  $^1\text{H-NMR}$  spectra of caecal contents from rats fed Chow vs LF (C) or Chow vs. HF/HS (D) or LF vs LF-I (H) diets. Metabolites are color-coded according their correlation coefficient, red indicating a very strong positive correlation. The direction of the metabolite indicates the group with which it is positively associated as labeled on the diagrams. BCAA: branched-chain amino acids, hpx: hypoxanthine, 4-HPPA: 4-hydroxyphenylacetate, uk: unknown compound.

Caecum tissue relative weight (I) and caecal IAP activity (J) in Chow, LF, LF-I, HF/HS and HF/HS-I-diet fed rats at week 8. Values are means  $\pm$  SEM (n=8 rats per group). Means with different letters are significantly different ( $P < 0.05$ ).

**Figure 3: Effect of diets on CCK satiation**

Intake expressed as actual energy intake (A, C) or as percentage of saline energy intake after 20 min (A, B) or from 20 to 60 min (C, D) after intraperitoneal injection of either saline (400  $\mu$ l) or CCK-8S at the lowest dose (1  $\mu$ g/kg body weight) or the highest dose (3  $\mu$ g/kg of body weight) of Chow (black), LF (grey), LF-I (striped grey), HF/HS (white) and HF/HS-I (dotted white) diet-fed rats.

Values are means  $\pm$  SEM (n=7 to 8 rats per group). Each rat received vehicle and the two CCK doses randomly. \* P<0.05 compared to saline in the same dietary group.

**Figure 4: Correlations between calorie intake after CCK low dose injection and adiposity parameters.**

Correlation between energy intake after CCK 1  $\mu$ g/kg and adiposity index (A), retroperitoneal fat pad relative weight (B), epididymal fat pad relative weight (C), percentage of large adipocytes in the retroperitoneal fat pad (D) and between decrease in energy intake after CCK 1  $\mu$ g/kg expressed in percent of intake under saline and retroperitoneal fat pad relative weight (E) or plasma LBP (F). Each point represents one animal: black circle: chow, grey square: LF, open square: LF-I, black triangle: HF/HS, open triangle: HF/HS-I.

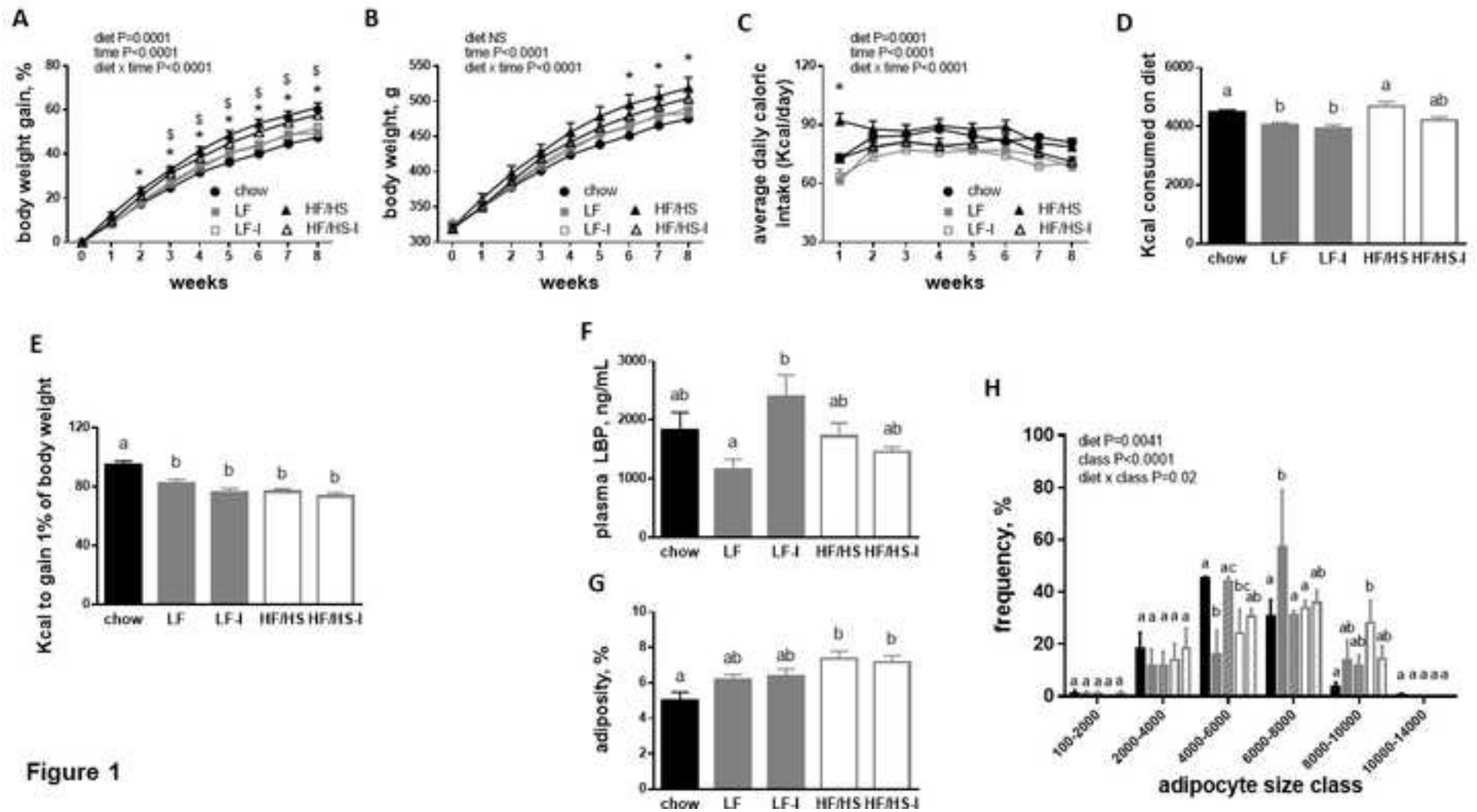


Figure 1

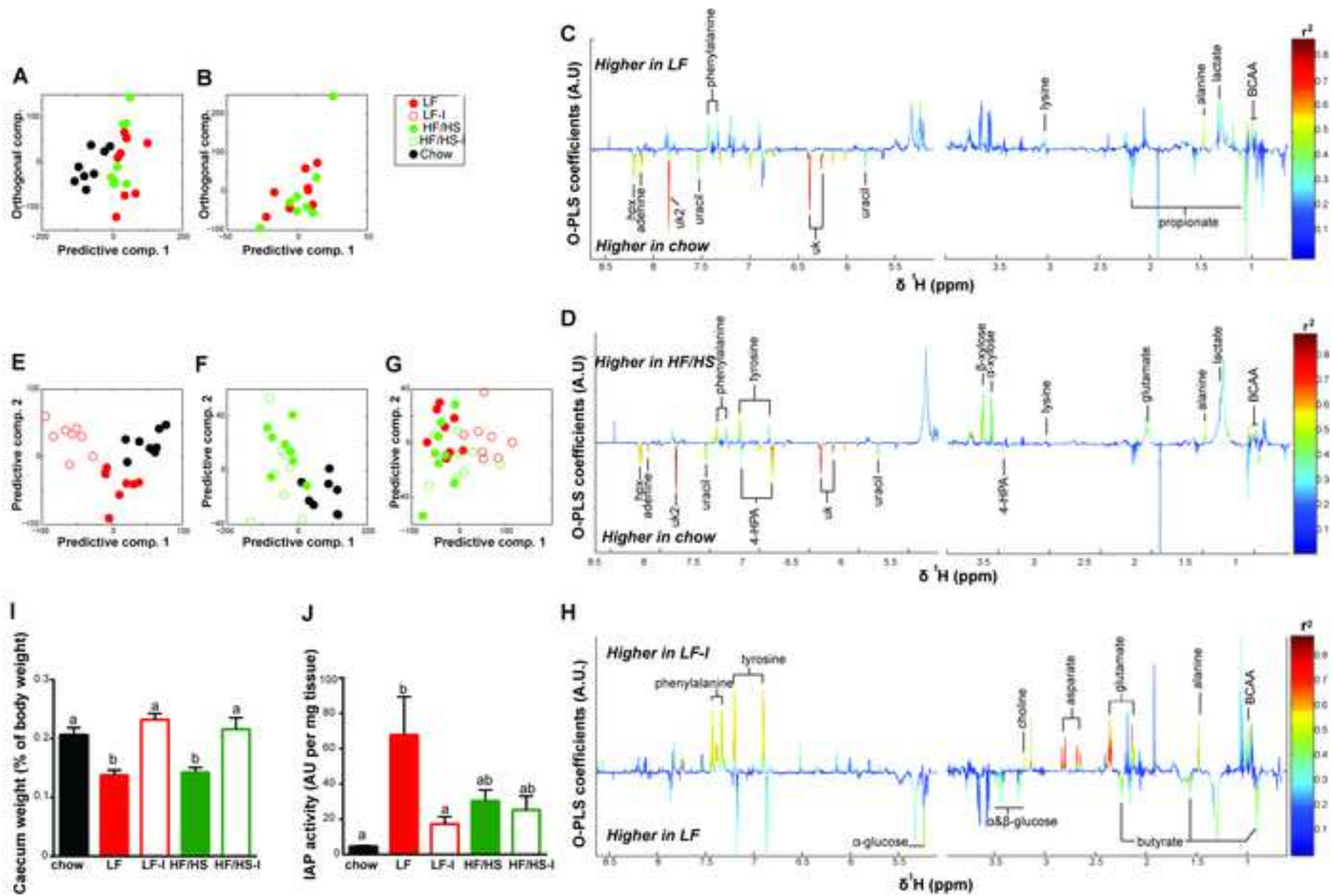




Figure 3

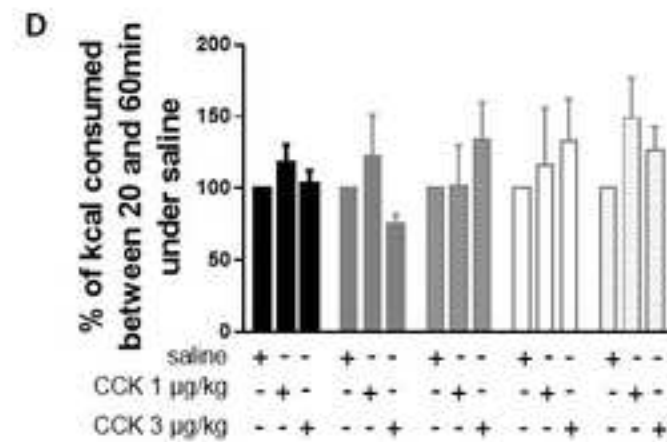
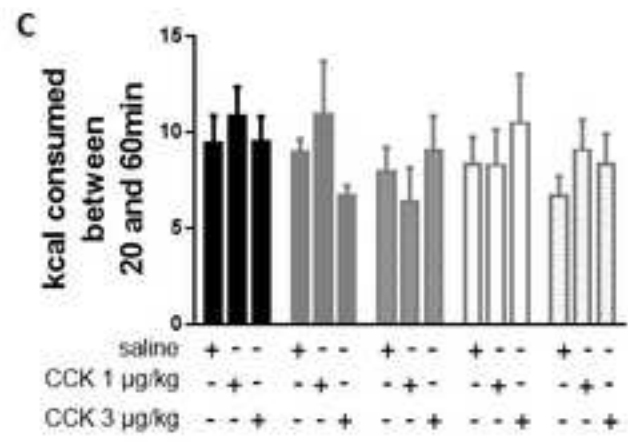
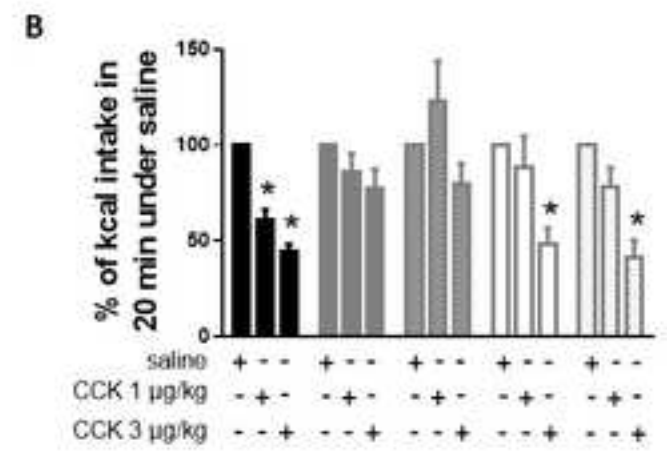
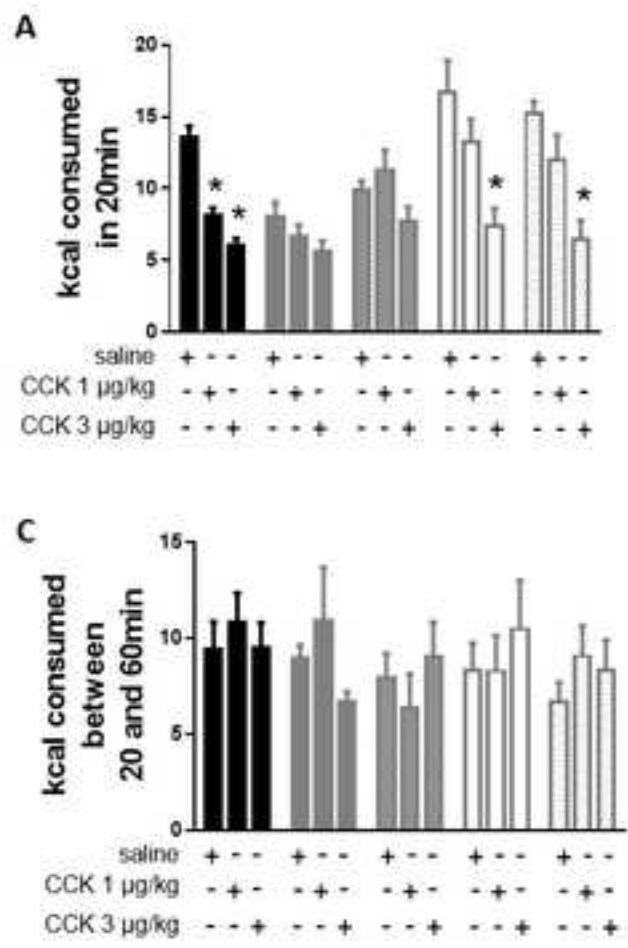
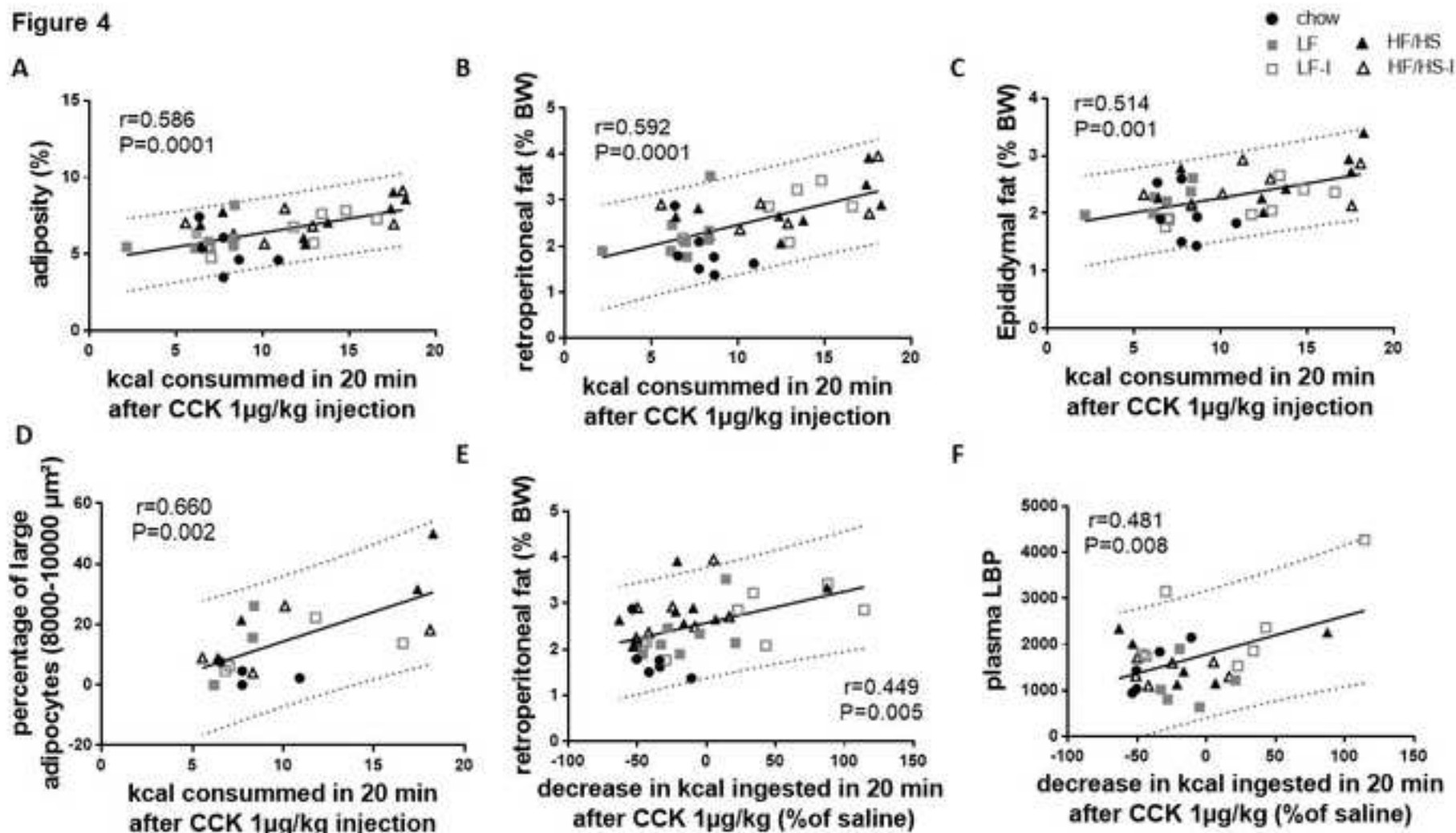


Figure 4



**Table 1: Ingredient composition and macronutrient contribution to energy of the chow diet.**

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***Ingredients :***

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Ground corn, dehulled soybean meal, dried beet pulp, fish meal, ground oats, brewers dried yeast, cane molasses, dehydrated alfalfa meal, dried whey, wheat germ, porcine animal fat, porcine meat meal, wheat midlings, salt, vitamin mixture, mineral mixture

***Energy (%)***

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Proteins	29
Lipids	13
Carbohydrates	58

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**Table 3: OPLS-DA model parameters and validation statistics calculated from <sup>1</sup>H NMR caecal content spectra.**

Model	Number of predictive components	Number of orthogonal components	R <sup>2</sup> X	R <sup>2</sup> Y	Q <sup>2</sup> Y	p-value <sup>1</sup>
Chow vs LF vs HF/HS	1	1	0.11	0.96	0.76	0.001
HF/HS vs LF	1	1	0.06	0.70	-0.08	0.52
Chow vs LF	1	1	0.13	0.97	0.67	0.001
Chow vs HF/HS	1	1	0.15	0.97	0.75	0.001
Chow vs LF vs LF-I	2	0	0.19	0.82	0.59	0.001
Chow vs HF/HS vs HF/HS-I	2	0	0.17	0.78	0.44	0.001
LF vs LF-I vs HF/HS vs HF/HS-I	2	1	0.11	0.86	0.34	0.001
HF vs HF/HS-I	1	0	0.07	0.76	0.08	0.26
LF vs LF-I	1	1	0.13	0.75	0.98	0.001

<sup>1</sup>Cumulative probability value determined at the 95<sup>th</sup> percentile to determine whether the result of the model was significantly different from the result calculated from 1000 random permutations of Y.

**Table 3: OPLS-DA model parameters and validation statistics calculated from <sup>1</sup>H NMR caecal content spectra.**

Model	Number of predictive components	Number of orthogonal components	R <sup>2</sup> X	R <sup>2</sup> Y	Q <sup>2</sup> Y	p-value <sup>1</sup>
Chow vs LF vs HF/HS	1	1	0.11	0.96	0.76	0.001
HF/HS vs LF	1	1	0.06	0.70	-0.08	0.52
Chow vs LF	1	1	0.13	0.97	0.67	0.001
Chow vs HF/HS	1	1	0.15	0.97	0.75	0.001
Chow vs LF vs LF-I	2	0	0.19	0.82	0.59	0.001
Chow vs HF/HS vs HF/HS-I	2	0	0.17	0.78	0.44	0.001
LF vs LF-I vs HF/HS vs HF/HS-I	2	1	0.11	0.86	0.34	0.001
HF vs HF/HS-I	1	0	0.07	0.76	0.08	0.26
LF vs LF-I	1	1	0.13	0.75	0.98	0.001

<sup>1</sup>Cumulative probability value determined at the 95<sup>th</sup> percentile to determine whether the result of the model was significantly different from the result calculated from 1000 random permutations of Y.

**Table 4. Changes in caecal content metabolites of rats fed LF, LF-I, HF/HS or HF/HS-I diets relative to chow diet (area under the curves of selected 1H-NMR peaks expressed as fold-change compared to chow).**

Metabolites	Diagnosti	Chow	LF	LF-I	HF/HS	HF/HS-I	P-Value
	c NMR shift in ppm (multiplici ty)						
Bile acid 1	0.66 (s)	1.00 ± 0.22	0.38 ± 0.10	0.74 ± 0.14	0.66 ± 0.21	1.31 ± 0.37	0.07
Bile acid 2	0.68 (s)	1.00 ± 0.22	0.73 ± 0.23	0.55 ± 0.11	0.79 ± 0.31	0.62 ± 0.13	0.67
Bile acid 3	0.7 (s)	1.00 ± 0.15	0.54 ± 0.09	0.63 ± 0.16	0.56 ± 0.20	0.74 ± 0.20	0.30
Bile acid 4	0.73 (s)	1.00 ± 0.20	1.05 ± 0.29	0.66 ± 0.18	0.62 ± 0.16	0.76 ± 0.18	0.92
Lactate	4.11 (q)	1.00 ± 0.07	0.96 ± 0.05	0.93 ± 0.07	0.98 ± 0.08	0.94 ± 0.08	0.95
Acetate	1.92 (s)	1.00 ± 0.10	0.74 ± 0.13	0.78 ± 0.12	0.74 ± 0.13	0.71 ± 0.11	0.42
Propionate	1.05 (t)	1.00 ± 0.07	0.71 ± 0.08	0.68 ± 0.13	0.96 ± 0.14	0.64 ± 0.10	0.06
Butyrate	0.9 (t)	1.00 ± 0.07 <sup>ab</sup>	0.95 ± 0.22 <sup>ab</sup>	1.42 ± 0.32 <sup>b</sup>	0.57 ± 0.03 <sup>a</sup>	0.86 ± 0.08 <sup>ab</sup>	<b>0.03</b>

Alanine	1.48 (d)	1.00 ± 0.09 <sup>a</sup>	1.30 ± 0.10 <sup>a</sup>	1.97 ± 0.16 <sup>b</sup>	1.44 ± 0.19 <sup>ab</sup>	1.93 ± 0.10 <sup>b</sup>	<b>&lt;0.0001</b>
Aspartate	2.38 (dd)	1.00 ± 0.07 <sup>a</sup>	1.32 ± 0.02 <sup>a</sup>	3.11 ± 0.25 <sup>c</sup>	1.67 ± 0.23 <sup>ab</sup>	2.17 ± 0.24 <sup>b</sup>	<b>&lt;0.0001</b>
Glutamate	2.36 (m)	1.00 ± 0.07 <sup>a</sup>	1.00 ± 0.07 <sup>a</sup>	1.76 ± 0.14 <sup>b</sup>	1.01 ± 0.11 <sup>a</sup>	1.14 ± 0.20 <sup>a</sup>	<b>0.0003</b>
Isoleucine	1.01 (d)	1.00 ± 0.10 <sup>a</sup>	2.56 ± 0.24 <sup>c</sup>	1.77 ± 0.12 <sup>b</sup>	2.32 ± 0.27 <sup>c</sup>	1.54 ± 0.13 <sup>b</sup>	<b>&lt;0.0001</b>
Leucine	0.96 (t)	1.00 ± 0.10	0.99 ± 0.14	0.71 ± 0.05	0.97 ± 0.16	0.77 ± 0.10	0.26
Lysine	3.03 (t)	1.00 ± 0.08 <sup>a</sup>	1.53 ± 0.21 <sup>b</sup>	1.65 ± 0.12 <sup>b</sup>	1.31 ± 0.09 <sup>ab</sup>	1.51 ± 0.07 <sup>b</sup>	<b>0.008</b>
Ornithine	1.7 (m)	1.00 ± 0.09	1.11 ± 0.11	1.28 ± 0.07	0.94 ± 0.09	1.15 ± 0.09	0.08
Phenylalanine	7.42 (t)	1.00 ± 0.12 <sup>a</sup>	1.39 ± 0.11 <sup>ab</sup>	2.10 ± 0.17 <sup>b</sup>	1.45 ± 0.06 <sup>ab</sup>	1.99 ± 0.16 <sup>b</sup>	<b>&lt;0.0001</b>
Taurine	3.43 (t)	1.00 ± 0.29	1.19 ± 0.23	0.68 ± 0.12	1.20 ± 0.36	1.43 ± 0.31	0.41
Tyrosine	6.9 (t)	1.00 ± 0.12 <sup>a</sup>	1.23 ± 0.09 <sup>ab</sup>	1.99 ± 0.17 <sup>b</sup>	1.29 ± 0.06 <sup>ab</sup>	1.83 ± 0.18 <sup>b</sup>	<b>&lt;0.0001</b>
Valine	0.99 (d)	1.00 ± 0.09 <sup>a</sup>	2.36 ± 0.36 <sup>b</sup>	1.38 ± 0.10 <sup>ab</sup>	1.75 ± 0.14 <sup>ab</sup>	1.29 ± 0.10 <sup>ab</sup>	<b>0.0001</b>
5-aminovalerate	2.24 (t)	1.00 ± 0.10 <sup>ab</sup>	1.48 ± 0.25 <sup>b</sup>	0.67 ± 0.12 <sup>a</sup>	1.08 ± 0.09 <sup>ab</sup>	1.14 ± 0.20 <sup>ab</sup>	<b>0.03</b>
α-keto-isovalerate	1.13 (d)	1.00 ± 0.08 <sup>a</sup>	0.48 ± 0.09 <sup>b</sup>	0.64 ± 0.13 <sup>ab</sup>	0.65 ± 0.14 <sup>ab</sup>	0.64 ± 0.09 <sup>ab</sup>	<b>0.03</b>
4-HPPA	6.86 (d)	1.00 ± 0.12 <sup>a</sup>	0.62 ± 0.28 <sup>a</sup>	0.14 ± 0.03 <sup>b</sup>	0.32 ± 0.08 <sup>ab</sup>	0.14 ± 0.02 <sup>b</sup>	<b>&lt;0.0001</b>
α-glucose	5.25 (d)	1.00 ± 0.13	1.54 ± 0.18	1.15 ± 0.15	1.56 ± 0.21	1.41 ± 0.21	0.13
β-glucose	4.65 (d)	1.00 ± 0.08 <sup>ab</sup>	1.31 ± 0.34 <sup>b</sup>	0.76 ± 0.07 <sup>a</sup>	0.93 ± 0.13 <sup>ab</sup>	1.11 ± 0.18 <sup>ab</sup>	<b>0.05</b>
succinate	2.4 (s)	1.00 ± 0.11	1.09 ± 0.39	1.26 ± 0.53	0.94 ± 0.22	1.95 ± 0.63	0.45

$\beta$ -hydroxybutyrate	1.25 (d)	1.00 $\pm$ 0.14 <sup>a</sup>	1.80 $\pm$ 0.63 <sup>ab</sup>	1.44 $\pm$ 0.27 <sup>ab</sup>	2.88 $\pm$ 0.63 <sup>b</sup>	2.24 $\pm$ 0.49 <sup>ab</sup>	<b>0.03</b>
$\alpha$ -keto-glutarate	2.45 (t)	1.00 $\pm$ 0.10 <sup>a</sup>	0.69 $\pm$ 0.13 <sup>ab</sup>	0.52 $\pm$ 0.03 <sup>ab</sup>	0.60 $\pm$ 0.09 <sup>ab</sup>	0.43 $\pm$ 0.05 <sup>b</sup>	<b>0.03</b>
N-acetyl group	2.05 (s)	1.00 $\pm$ 0.14	1.32 $\pm$ 0.25	1.35 $\pm$ 0.16	1.68 $\pm$ 0.20	1.47 $\pm$ 0.21	0.19
Trimethylamine	2.9 (s)	1.00 $\pm$ 0.08 <sup>a</sup>	0.79 $\pm$ 0.07 <sup>ab</sup>	0.84 $\pm$ 0.07 <sup>ab</sup>	0.69 $\pm$ 0.08 <sup>b</sup>	0.71 $\pm$ 0.08 <sup>ab</sup>	<b>0.04</b>
Choline	3.2 (s)	1.00 $\pm$ 0.16 <sup>ab</sup>	0.81 $\pm$ 0.09 <sup>a</sup>	1.57 $\pm$ 0.22 <sup>b</sup>	0.84 $\pm$ 0.01 <sup>a</sup>	1.05 $\pm$ 0.12 <sup>ab</sup>	<b>0.005</b>
Uracil	5.8 (d)	1.00 $\pm$ 0.10	0.69 $\pm$ 0.08	0.85 $\pm$ 0.11	0.59 $\pm$ 0.11	0.66 $\pm$ 0.13	0.07
Hypoxanthine	8.19 (s)	1.00 $\pm$ 0.09 <sup>a</sup>	0.63 $\pm$ 0.06 <sup>b</sup>	0.76 $\pm$ 0.07 <sup>ab</sup>	0.50 $\pm$ 0.07 <sup>b</sup>	0.54 $\pm$ 0.08 <sup>b</sup>	<b>0.0003</b>
Unknown 1	6.38 (s)	1.00 $\pm$ 0.12 <sup>a</sup>	0.14 $\pm$ 0.02 <sup>b</sup>	0.13 $\pm$ 0.03 <sup>b</sup>	0.10 $\pm$ 0.01 <sup>b</sup>	0.14 $\pm$ 0.02 <sup>b</sup>	<b>&lt;0.0002</b>
Unknown 2	6.70 (m)	1.00 $\pm$ 0.11	0.87 $\pm$ 0.40	0.61 $\pm$ 0.20	1.74 $\pm$ 0.88	0.85 $\pm$ 0.21	0.47
Unknown 4	6.38 (m)	1.00 $\pm$ 0.12 <sup>a</sup>	0.09 $\pm$ 0.01 <sup>b</sup>	0.09 $\pm$ 0.02 <sup>b</sup>	0.07 $\pm$ 0.01 <sup>b</sup>	0.09 $\pm$ 0.02 <sup>b</sup>	<b>&lt;0.0001</b>
Ethanol	1.19 (t)	1.00 $\pm$ 0.05	0.95 $\pm$ 0.19	0.99 $\pm$ 0.08	1.30 $\pm$ 0.30	1.29 $\pm$ 0.12	0.41
MCFA	1.33 (br)	1.00 $\pm$ 0.11	1.60 $\pm$ 0.27	0.75 $\pm$ 0.10	3.35 $\pm$ 1.31	2.02 $\pm$ 0.83	0.10

d: doublet, dd: doublet of doublet, t: triplet, s: singulet, m: multiplet, 4-HPPA: 4-Hydroxyphenylacetate, MCFA: medium-chain fatty acids.

<sup>1</sup> P-value of the ANOVA testing the diet effect. a, b, c: P<0.05 after Tukey post-hoc tests. Data are means  $\pm$  SEM. n=8 rats/group



**Table 5. Change in PTP1b level in nodose ganglia after leptin injection in rats fed chow, LF, LF-I, HF/HS or HF/HS-I diets.**

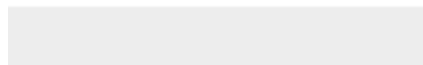
	saline	leptin
Chow diet	0.64 ± 0.22	1.46 ± 0.46
LF diet	0.52 ± 0.42	0.71 ± 0.18
LF-I diet	0.76 ± 0.18	0.76 ± 0.26
HF/HS diet	0.58 ± 0.21	0.42 ± 0.09
HF/HS-I diet	1.10 ± 0.17	0.22 ± 0.09 *

Values are means ± SEM (n=2 to 4 rats per group, \* P<0.05 compared to saline)



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Suppl Fig1.tif





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