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Reversion to a control balanced diet is able to restore body weight and to recover altered metabolic parameters in adult rats long-term fed on a cafeteria diet

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ABSTRACT

The increased intake of fat-rich foods is one of the causes of the increasing incidence of obesity. However, there are controversial data on the reversibility of diet-induced obesity and its metabolic complications when adopting a control energy-balanced diet. Our aim was to evaluate the ability to reset not only body weight but also metabolic disorders caused by a highly palatable high fat diet, cafeteria diet, administered to adult rats, when replaced by a control diet (post-cafeteria model). Four-months of cafeteria diet-feeding produced important metabolic alterations in comparison to a commercial purified high fat diet: a rapid, drastic increase in body weight, adiposity and related complications such as insulin resistance, decreased glucose tolerance and development of hepatic steatosis. At gene level, decreased lipogenic and increased lipolytic gene expression in key energy homeostatic tissues as a physiological adaptation to increased fat intake was observed. In addition, fasting response of serum parameters and of key genes in lipid metabolism was impaired in cafeteria-fed animals. Contrary to what we have previously described if cafeteria diet is administered early in life, when administered to adult animals, its replacement with a balanced diet is able to restore body weight. Cafeteria diet withdrawal also allows recovery from metabolic damage, gene expression regulation and fasting response, the degree of which is dependent on the time of exposure to the cafeteria diet. In conclusion, adherence to an *ad libitum* intake of a balanced standard diet can enable the recovery of healthy status in animals which were previously exposed to an unhealthy cafeteria diet in adult age.

1. Introduction

The prevalence of obesity caused by environmental factors has increased exponentially in developed countries due to consumption of energy rich foods, often with a high fat content, and by a reduction in physical activity (Schrauwen & Westerterp, 2000; Wright & Aronne, 2012). Rodent animal models have been traditionally used to study obesity induced by the intake of high fat diets (Buettner, Scholmerich, & Bollheimer, 2007) which allows obtaining metabolic information not attainable with non-invasive sampling. These diets result in increased body weight and adiposity and, as happens in humans, in other alterations related to metabolic syndrome such as insulin resistance, hypertriglyceridemia and hypercholesterolemia (Sampey et al., 2011). In addition, high fat content diets induce metabolic changes in different tissues involved in energy homeostasis control as a physiological adaptation to the increased amount of fat (Estadella, Oyama, Dâmaso, Ribeiro, & Oller Do Nascimento, 2004). Among the different experimental diets used to induce obesity, the so-called cafeteria diet is a highly palatable hyperlipidic diet that induces persistent voluntary hyperphagia and a rapid, high and persistent weight gain (Caimari, Oliver, Rodenburg, Keijer, & Palou, 2010b; Sampey et al., 2011). In contrast, commercial high fat diets produce an initial high energy intake, associated to early weight gain, but this overfeeding declines after some weeks, and body weight is then stabilized (Sampey et al., 2011; Vial et al., 2011). Remarkably, although both diets are associated with the development of obesity and related alterations, they trigger different degrees of overweight/obesity (Caimari, Oliver, et al., 2010b; Priego, Sánchez, Palou, & Picó, 2009; Sampey et al., 2011). To our knowledge there is only one recent study comparing directly the two hyperlipidic diets (Sampey et al., 2011). A question remains as to whether it is possible to reverse increased body weight and adiposity and the important

Abbreviations: *Atgl*, adipose triglyceride lipase; AUC, area under the curve; BAT, brown adipose tissue; BMI, body mass index; CAF, cafeteria; *Cpt1a*, carnitine palmitoyltransferase 1a; *Cpt1b*, carnitine palmitoyltransferase 1b; eWAT, epididymal white adipose tissue; *Fasn*, fatty acid synthase; *Fgf21*, fibroblast growth factor 21; *Gdi1*, guanosine diphosphate dissociation inhibitor 1; HF, high fat; HOMA-IR, homeostatic model assessment for insulin resistance; iWAT, inguinal white adipose tissue; mWAT, mesenteric white adipose tissue; OGTT, oral glucose tolerance test; PCAF, post-cafeteria; rWAT, retroperitoneal white adipose tissue; *Srebp1a*, sterol regulatory element-binding protein 1a.

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metabolic alterations caused by the prolonged intake of a cafeteria diet. Our previous studies indicate that prolonged (3 months) cafeteria diet feeding initiated during early life (from the age of 10 days) produces an important and persistent increase in body weight and permanent metabolic alterations which are not completely reverted when this diet is replaced by a control normolipidic diet (post-cafeteria model) (Lladó, Proenza, Serra, Palou, & Pons, 1991; Matamala et al., 1996; Proenza et al., 1992; Rodríguez et al., 2003). However, the consequences of reversion to a control diet in animals with prolonged cafeteria diet feeding during adulthood have not been addressed in detail. Different studies have analyzed the effects of the cafeteria diet withdrawal in rodents, but the results are controversial; some indicate persistence of obesity (Rolls, Rowe, & Turner, 1980; South, Westbrook, & Morris, 2012) while others report more or less reversal of obesity (Levin & Dunn-Meynell, 2002; Ong, Wanasuria, Lin, Hiscock, & Muhlhausler, 2013). However, most of the studies cover only a few general metabolic or anthropometric parameters or are focused in behavioral aspects, therefore a detailed analysis to determine the success of cafeteria diet removal not only on recovery of body weight but also on recovery of alterations related to metabolic syndrome, energy homeostasis maintenance or altered gene expression have not been performed.

Thus, the aim of this study was to evaluate whether a reversion to a standard balanced diet is enough to recover body weight and the altered metabolic parameters in adult animals fed on a cafeteria diet for different time periods. Since impaired fasting response is an indicator of metabolic alterations related to obesity (Caimari, Oliver, & Palou, 2007), we have studied recovery of fasting sensitivity to evaluate metabolic recovery associated to weight loss. Secondarily, we have compared the impact of a long-term cafeteria and a commercial high fat diet feeding on body weight and energy homeostatic metabolism.

2. Methods and materials

2.1. Animals, diets and experimental design

All animal experimental procedures were reviewed and approved by the Bioethical Committee of our university, and guidelines for the use and care of laboratory animals of the university were followed.

Two different experimental designs were performed. In Experiment 1, two-month-old male Wistar rats (Charles River Laboratories España, SA, Barcelona, Spain) were divided into groups fed for 4 months with different diets: a control group (Control; n = 12), with animals fed a standard normal fat diet – control diet – (with 10% calories from fat) (Ref. D12450B, Research Diets, Brogaarden, Denmark); a cafeteria group (CAF1; n = 12), with animals fed a cafeteria diet in addition to the control diet; a post-cafeteria group (PCAF1; n = 12), with animals fed initially for two months with cafeteria diet plus control diet, and for two more months with a control diet; and a high fat group (HF; n = 7), with animals fed a commercial high fat diet (with 60% calories from fat) (Ref. D12492, Research Diets, Brogaarden, Denmark). In Experiment 2, two-month-old male Wistar rats were divided into groups fed for 3 months with different diets: a control group (Control; n = 12), with animals fed a control normal fat diet; a cafeteria group (CAF2; n = 12), with animals fed a cafeteria diet in addition to the control diet; and a post-cafeteria group (PCAF2; n = 12), with animals fed for one and a half months with a cafeteria diet in addition to the control diet, and then for one and a half months with the control diet.

The cafeteria diet contained the following foodstuffs: cookies with liver pate and sobrassada (a typical Majorcan sausage), candies, fresh bacon, biscuits, chocolate, salted peanuts, cheese, milk containing 20% (w/v) sucrose and ensaïmada (a typical Majorcan pastry) (Caimari, Oliver, et al., 2010b; Oliver, Reynés, Caimari, & Palou, 2013). The gross composition of the diets was as follows: for the control normal fat diet, 70% calories from carbohydrate, 10% from fat and 20% from protein; for the cafeteria diet, 27% calories from carbohydrate, 62% from fat and 11% from protein (this composition was calculated based on the foodstuffs ingested by the animals; Ribot, Rodriguez, Rodriguez, & Palou, 2008); and for the commercial high fat diet, 20% calories from carbohydrate, 60% from fat and 20% from protein. Fatty acid profile (saturated/unsaturated) was similar for the two-hyperlipidic diets used.

The animals were housed in pairs at 22 °C with a period of light/dark of 12 h. Food and water were offered *ad libitum* and body weight was followed twice a week. Food intake was recorded in the control and HF groups (in Experiment 1) and in PCAF groups (in Experiments 1 and 2); an exhaustive recording of the intake of the cafeteria diet used in this experiment had already been performed by our group (Ribot et al., 2008). Body fat composition was determined every two weeks using an EchoMRI-700™ (Echo Medical Systems, LLC, TX, USA). At the end of the experimental period, when animals were 6 or 5 months old (for Experiments 1 and 2 respectively), each group of rats was divided into two subgroups and submitted to different feeding conditions (n = 6 for each condition): feeding (animals provided with *ad libitum* access to diet) and fasting (animals with a nocturnal fasting of 14 h). Cages were changed immediately prior to food deprivation in order to prevent coprophagy.

After the experimental feeding period, fed and fasted animals were weighed and their nasal–anal length was measured to obtain the body mass index and the Lee index. Afterwards, the animals were sacrificed by decapitation and the different white adipose tissue depots, both visceral (epididymal, mesenteric and retroperitoneal) and subcutaneous (inguinal), interscapular brown adipose tissue, liver and gastrocnemius muscle were rapidly removed, weighed, frozen in liquid nitrogen and stored at −80 °C until RNA analysis. Troncular blood was collected from the neck, stored at room temperature for 1 h, and was then centrifuged at 1000 *g* for 10 min at 4 °C to collect the serum.

2.2. Adiposity index

Adiposity was determined as an adiposity index computed for each rat: the sum of epididymal, inguinal, mesenteric and retroperitoneal white adipose tissue depots was weighed and expressed as a percentage of total body weight.

In addition, two other indicators of adiposity were calculated: the body mass index (BMI) and the Lee index (Novelli et al., 2007; Scoaris et al., 2010).

2.3. Measurement of circulating parameters (glucose, insulin, leptin, ghrelin and triacylglycerols)

Blood glucose concentration was measured using an Accu-Chek Glucometer (Roche Diagnostics, Barcelona, Spain). Serum insulin and leptin levels were determined using enzyme-linked immunosorbent assay (ELISA) kits (from Mercodia AB, Uppsala, Sweden and R&D Systems, Minneapolis, MN, USA, respectively). Serum ghrelin concentration was measured using an enzyme immunoassay kit (Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA). Circulating triacylglycerols were measured using a commercial enzymatic colorimetric kit (Sigma Diagnostics, St. Louis, MO, USA).

2.4. HOMA-IR analysis

Insulin resistance was assessed by the homeostatic model assessment for insulin resistance (HOMA-IR). It was calculated from fasting insulin and glucose concentrations using the formula of Matthews et al. (1985).

2.5. Oral glucose tolerance test

Glucose responses to an oral glucose tolerance test (OGTT) were also measured to assess insulin sensitivity. The test was performed in animals of Experiment 1, at the end of the experimental period (6-month-old animals), as previously described by Sánchez et al.