Original article

MOLECULAR METABOLISM

Obesogenic memory can confer long-term increases in adipose tissue but not liver inflammation and insulin resistance after weight loss

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ABSTRACT

Objective: Obesity represents a major risk factor for the development of type 2 diabetes mellitus, atherosclerosis and certain cancer entities. Treatment of obesity is hindered by the long-term maintenance of initially reduced body weight, and it remains unclear whether all pathologies associated with obesity are fully reversible even upon successfully maintained weight loss.

Methods: We compared high fat diet-fed, weight reduced and lean mice in terms of body weight development, adipose tissue and liver insulin sensitivity as well as inflammatory gene expression. Moreover, we assessed similar parameters in a human cohort before and after bariatric surgery.

Results: Compared to lean animals, mice that demonstrated successful weight reduction showed increased weight gain following exposure to *ad libitum* control diet. However, pair-feeding weight-reduced mice with lean controls efficiently stabilized body weight, indicating that hyperphagia was the predominant cause for the observed weight regain. Additionally, whereas glucose tolerance improved rapidly after weight loss, systemic insulin resistance was retained and ameliorated only upon prolonged pair-feeding. Weight loss enhanced insulin action and resolved pro-inflammatory gene expression exclusively in the liver, whereas visceral adipose tissue displayed no significant improvement of metabolic and inflammatory parameters compared to obese mice. Similarly, bariatric surgery in humans ($n = 55$) resulted in massive weight reduction, improved hepatic inflammation and systemic glucose homeostasis, while adipose tissue inflammation remained unaffected and adipocyte-autonomous insulin action only exhibit minor improvements in a subgroup of patients (42%).

Conclusions: These results demonstrate that although sustained weight loss improves systemic glucose homeostasis, primarily through improved inflammation and insulin action in liver, a remarkable obesogenic memory can confer long-term increases in adipose tissue inflammation and insulin resistance in mice as well as in a significant subpopulation of obese patients.

Keywords Obesity; Weight loss; Weight regain; Insulin resistance; Metabolic inflammation

1. INTRODUCTION

Obesity is a major health problem with increasing prevalence around the world [1,2]. The World Health Organization estimates that each year, 2.8 million people die as a result of being overweight or obese [3]. The increased mortality can mainly be attributed to obesity-associated diseases such as type 2 diabetes mellitus (T2DM), cardiovascular disease, and some types of cancer [4]. Weight reduction represents an obesity intervention strategy, which efficiently ameliorates some associated diseases [5–7].

To successfully promote weight loss, energy intake has to fall below total energy expenditure. Increasing energy expenditure by physical exercise improves several obesity-related disorders and should therefore be an important component of any weight loss plan. However, physical exercise alone, without concomitant dietary changes, promotes only modest weight reduction [8,9], and, importantly, only few patients successfully manage to include exercise into their daily schedule. Therefore, decreasing caloric intake remains the single most effective and cost efficient intervention strategy for obesity. Notably, for the beneficial effects, it is of little importance what exact dietary

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composition is used during caloric restriction, as long as weight reduction can be achieved [10,11]. However, intensive lifestyle changes that lead to weight reduction do not necessarily reduce cardiovascular mortality in obese adults with T2DM [12]. It is therefore questionable whether weight loss is sufficient to reverse all metabolic or inflammatory changes induced by severe obesity. Moreover, although weight loss programs can result in an average body weight reduction of 10% [13], the majority of patients regain their body weight quickly [14,15], once again placing them at high risk to develop obesity-associated diseases.

The biological mechanisms underlying this key obstacle in the treatment of obesity are poorly understood. There is evidence from human studies suggesting that weight reduction ultimately leads to a profound decrease in energy expenditure [16–19]. However, whether this decrease is more than what can be explained by the changes in body composition remains controversial [20]. A disadvantage of human studies designed to assess the mechanism for weight regain is the selection bias of choosing already obese patients. Genetic, epigenetic and behavioral predispositions that led to obesity development in the first place influence weight regain and might also be responsible for some of the differences observed after weight reduction [21].

Rodent models offer the advantage of genetic similarity and the possibility to better control nutrient composition of the diet and caloric intake. However, there are only a limited number of rodent studies published so far that analyze the long-term consequences of weight loss and regain. Studies that used obesity-prone rats or embryonic glutamate injections to induce obesity fail to distinguish between the direct effects of weight reduction in obesity and changes of the animal model itself [22,23]. Thus, the biological mechanisms behind weight regain as well as the long-term consequences of weight loss on tissue-specific insulin action and insulin secretion remain only partly understood.

In the present study, we have analyzed the effect of caloric restriction in HFD-induced obesity in mice. Our experiments revealed that weight reduction efficiently improved systemic glucose tolerance and that insulin sensitivity was improved in the liver upon weight loss but did not equally improve in adipose tissue after weight reduction. Consistently, while obesity-induced hepatic inflammation resolved upon weight loss, adipose-tissue inflammation persisted. Importantly, we also demonstrate that upon massive and sustained weight loss in humans undergoing bariatric surgery, hepatic inflammation resolves, while at least in a subgroup of obese individuals, adipose tissue inflammation persists up to 12 months of follow-up. Furthermore, adipocyte-autonomous insulin action only marginally improves despite massive improvements of systemic insulin-induced glucose disposal in these patients. These results highlight that even successful, long-term weight reduction does not consistently reverse adverse, inflammatory reactions in adipose tissue associated with obesity, thus offering a molecular basis of sustained increased cardiovascular risk despite weight reduction.

2. METHODS

2.1. Animal procedures

All animal procedures and euthanasia were reviewed by the animal care committee and approved by local government authorities (Tierschutzkommission acc. §15 TSchG of the Landesamt für Natur, Umwelt und Verbraucherschutz North Rhine Westphalia) and were in accordance with NIH guidelines. Male C57BL/6 mice were acquired at 3 weeks age from Charles River Laboratory and held in a virus-free facility at 22–24 °C on a 12-h light, 12-h dark cycle. After one week of acclimatization in our animal facility, the mice were randomly assigned either a high fat diet (D12492, 60% kcal from fat, Research diets, New Brunswick, NJ, USA) or a sucrose-matched, low fat diet (D12450 J, 10% kcal from fat, Research diets, New Brunswick, NJ, USA). Body weight was measured weekly.

At 22 weeks of age, two thirds of the high fat diet-fed mice were switched to 40% caloric restriction using a caloric restriction diet (D11063001, 10% kcal of fat, Research diets, New Brunswick, NJ, USA). This diet was added to the experimental paradigm in order to ensure an equal intake of vitamins, minerals, and amino acids during the different dietary conditions, even when restricted to 60% of their prior caloric intake. For caloric restriction and pair-feeding, the rationed food was introduced once daily before the beginning of the night cycle.

2.2. Indirect calorimetry

Indirect calorimetry was performed for 120 h using an open-circuit, indirect calorimetry system including spontaneous activity by beam breaking (PhenoMaster System, TSE systems) as previously described [24].

2.3. Analysis of body composition by computer tomography

Imaging of mice was performed with a LaTheta LCT-100 (Aloka Co. LTD., Tokyo, Japan) micro computed tomography scanner. The X-ray source tube voltage was set at 50 kV with a constant 1 mA current. A holder with an inner diameter of 48 mm was used, resulting in pixel resolutions of 100 μm. Pitch was 0.5 mm and scan speed 4.5 s/slice. LaTheta software V2.10 estimates the contrast of the different tissues using differences in X-ray density. A Midazolam (5 mg/kg) and Medetomidine (0.5 mg/kg) anesthesia was used to immobilize the animals. After the procedure the anesthetics were antagonized using Flumazenil (0.5 mg/kg) and Atipamezole (2.5 mg/kg).

2.4. Glucose and insulin tolerance testing

Glucose tolerance testing (GTT) was performed in 6 h starved animals. After determination of the fasting blood glucose values, a 20% glucose solution (Glucosteril, Fresenius Kabi, Bad Homburg, Germany) was injected intraperitoneally at a dose of 10 ml/kg body weight. Blood glucose concentrations were determined at 15, 30, 60, and 120 min after injection (Bayer Contour, Bayer diabetes care, Leverkusen, Germany). For insulin tolerance testing (ITT), blood glucose values were measured in random fed mice before injecting 0.75 U/kgBW of insulin (Actrapid; Novo Nordisk A/S, Denmark), and blood glucose was measured at 15, 30 and 60 min after insulin injection.

2.5. Glucose-stimulated insulin secretion

Mice were fasted for 12 h. Blood samples were collected from mice before an i.v. injection by tail vein of 2 mg g^{-1} body weight of glucose (20% glucose; Glucosteril, Fresenius Kabi, Bad Homburg, Germany). Additional blood samples were collected 2, 5, 15, 30 and 60 min after the injection. Serum insulin levels were determined by enzyme-linked immunosorbent assay according to the manufacturer's instructions (mouse/rat Insulin ELISA, Crystal Chem Inc., Downers Grove, IL, USA).

2.6. Human bariatric surgery intervention study

We selected 23 Caucasian obese patients (14 women, 9 men) out of a group of 55 patients who underwent a two-step bariatric surgery strategy with gastric sleeve resection as the first step and a Roux-en-Y gastric bypass as second step 12 ± 2 months later (Table 1). At both time points, serum/plasma samples, omental and subcutaneous (sc) adipose tissue (AT) and liver biopsies were obtained. Patient selection was based on the absence (n = 23) of significant changes in the

