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Quinoa extract enriched in 20-hydroxyecdysone affects energy homeostasis and intestinal fat absorption in mice fed a high-fat diet

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HIGHLIGHTS

- We examine the effect of high fat feeding and 20-hydroxyecdysone on energy intake and energy expenditure in mice.
- Fecal lipid excretion was evaluated in 20-hydroxyecdysone treated mice.
- 20-Hydroxyecdysone intake modifies the glucose oxidation in vivo.
- Both increased energy expenditure and decreased lipid absorption are proposed for an anti-obesity effect of 20-hydroxyecdysone.

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ABSTRACT

In a previous study, we have demonstrated that a supplementation of a high-fat diet with a quinoa extract enriched in 20-hydroxyecdysone (QE) or pure 20-hydroxyecdysone (20E) could prevent the development of obesity. In line with the anti-obesity effect of QE, we used indirect calorimetry to examine the effect of dietary QE and 20E in high-fat fed mice on different components of energy metabolism. Mice were fed a high-fat (HF) diet with or without supplementation by QE or pure 20E for 3 weeks. As compared to mice maintained on a low-fat diet, HF feeding resulted in a marked physiological shift in energy homeostasis, associating a decrease in global energy expenditure (EE) and an increase in lipid utilization as assessed by the lower respiratory quotient (RQ). Supplementation with 20E increased energy expenditure while food intake and activity were not affected. Furthermore QE and 20E promoted a higher rate of glucose oxidation leading to an increased RQ value. In QE and 20E-treated HFD fed mice, there was an increase in fecal lipid excretion without any change in stool amount. Our study indicates that anti-obesity effect of QE can be explained by a global increase in energy expenditure, a shift in glucose metabolism towards oxidation to the detriment of lipogenesis and a decrease in dietary lipid absorption leading to reduced dietary lipid storage in adipose tissue.

1. Introduction

The maintenance of weight in adulthood requires that daily energy intakes be equivalent to the amounts of energy expended per day. Many studies have reported the effects of diets high in fat on energy balances [1]. Generally, it is well established that feeding a high fat diet leads to an excess of energy intake and contributes to the onset of obesity in animals and humans [2,3]. Recently, we have demonstrated that the chronic consumption of a quinoa extract alleviated the effect of high-fat feeding on adipose tissue gain [4]. This anti-obesity effect occurred without modification of food intake raising a possible effect on energy output at the metabolic and/or intestinal levels.

To study energy balance in small animals, modern systems can simultaneously measure multiple parameters such as food intake (FI), physical activity (Act), fecal and urine excretion, oxygen consumption (VO_2) and carbon dioxide production (VCO_2) [5,6]. VO_2 and VCO_2 are used to calculate energy expenditure (EE), and the ratio of VCO_2/VO_2 otherwise known as the respiratory quotient (RQ), can be used to estimate fuel utilization [7,8]. Revealing a defective energy balance (EB) by comparing FI and EE is quite impossible because the error in daily EB that leads to overweight in the long-term is very tiny (1–3%) and below the precision that can be obtained in the measurement of both

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EE and FI for which cumulative errors are close to 5% at best [6]. In addition, the period of measurement that is permitted by this technique (3–5 days) is usually below the normal window where FI and EE are closely matched (at least 7 days). However, for EB to be achieved, the fuel mix oxidized by the body should be equivalent to the nutrient content of the diet and this can be estimated by comparing the RQs to the food quotient (FQ), food quotient being the RQ expected from complete oxidation of the macronutrients in the diet.

Studies of metabolic responses to nutrient intake have shown that carbohydrate oxidation is correlated positively with daily variations in food intake, matching carbohydrate intake closely. By contrast, fat oxidation adapts more slowly and less completely to increased fat intake, in particular in obesity-prone subjects [9–11]. Thus fundamental differences in the control of carbohydrate and fat metabolism must be taken into account to understand body weight regulation and, particularly, in the context of resistance to diet-induced obesity [12]. On the other hand, the energy balance can be affected by the efficiency of dietary fat absorption, a parameter that can be modulated by the fat content in the diet and consequently plays an important role in energy balance by modulating the fecal energy output [13].

Quinoa (*Chenopodium quinoa*) is a pseudo-cereal which was consumed by Indians for a long time. It was recently rediscovered by food industries due to its protein quality. It is also an important source of minerals and vitamins, and has also been found to contain compounds like flavonoids with possible nutraceutical benefits [14]. Interestingly, quinoa is one of the rare food plants containing phytoecdysteroids, which display a wide array of pharmacological effects on mammals [15,16]. Although ecdysteroids are steroid hormones controlling molting and reproduction of arthropods, there is some evidence of their anabolic effects [17–19]. In addition, some studies have reported that the most common and abundant ecdysteroid, 20-hydroxyecdysone (20E), plays a role in immunomodulation, in the control of glucose homeostasis and more recently in the prevention of diet-induced obesity in mice [4,20–22].

The present study was intended to investigate the effect of a chronic quinoa extract intake in the regulation of energy homeostasis in mice fed a high-fat diet. Indirect calorimetry was used to know whether 1) an increase in fat intake modified the components of energy expenditure, and 2) the supplementation by 20-hydroxyecdysone in high-fat diet modified total energy expenditure, nutrient oxidation and spontaneous activity, as well as fecal lipid excretion.

2. Methods and procedures

2.1. Animals and diets

Six-week-old male C57BL/6J mice (Harlan Laboratories, Gannat, France) were used. The mice had free access to food and water and were housed in a controlled environment with a 12-h light–dark cycle and constant temperature (22 °C). In a first study, mice were fed a chow diet for the first week and then assigned to either a low fat (LF) or high fat (HF) diet for 3 weeks. The LF and HF diets contained 9% and 51% fat as energy (Table 1). In study 2, after the adaptation period on a chow diet, mice were fed a high fat diet (HF) supplemented or not with either quinoa extract (HFQ) or pure 20E (HF20E) for 3 weeks. Taking into account food intake and 20E content in quinoa extract, the amount of quinoa powder was adjusted to 0.28% accordingly to provide a similar dose of 20E (6 mg/day/kg body weight). Body weight was recorded weekly throughout the experimental period. Food intake was measured 3 times a week during the experimental period showing an equivalent energy intake within each experimental group. At the end of the experiment, mice were housed in individual metabolic chambers and VO_2, VCO_2, feeding pattern and spontaneous activity were recorded during 48 h (see below). Food intake was measured by weighing the food at the beginning and at the end of each 24 h period. At the end of the study, mice were anesthetized and sacrificed by collecting intracardiac blood in EDTA. Epididymal and inguinal adipose tissues were removed and weighed. Blood was centrifuged and the plasma was collected and stored at −20 °C until further determinations. All animal protocols have been submitted to the local ethics committee and have been subsequently authorized.

Table 1
Experimental diet composition and relative macronutrient caloric values.

	LF	HF	HFQ	HF20E
Weight (g/kg)				
Milk proteins concentrate[a]	140.0	170.0	170.0	170.0
Starch	622.4	360.0	360.0	360.0
Sucrose	100.3	57.0	57.0	57.0
Lard	–	235.0	235.0	235.0
Soybean oil	40.0	40.0	40.0	40.0
Mineral mixture	35.0	62.5	62.5	62.5
Vitamin mixture	10.0	12.5	12.5	12.5
Cellulose	50.0	62.5	62.5	62.5
Choline	2.3	2.3	2.3	2.3
QE	–	–	2.8	–
20E	–	–	–	0.0535
Energy (% kcal)				
Protein	15	14		
Carbohydrate	76	35		
Lipid	9	51		
	Energy density (kcal/g)			
	3.81	4.81		

LF, low fat diet; HF, high fat diet; QE, quinoa extract; 20E, 20-hydroxyecdysone.
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2.2. Quinoa extract preparation

The quinoa extract was prepared from seeds as previously described [4]. Briefly, after boiling for 5 min in water under shaking, the seeds were then boiled with water–ethanol (50–50 v/v) for 20 min. After filtration, the supernatant was dried under vacuum. The residue was then dissolved in absolute ethanol and ground using an Ultra-Turrax T-25 homogenizer (Janke and Kunkel, IKA® Labortechnik, Staufen, Germany). After centrifugation, the supernatant was dried in a rotary evaporator and finally ground into a fine powder. An aliquot of this powder was dissolved in water–methanol (65/35; v/v) and its 20-hydroxyecdysone (20E) content determined by HPLC. Using this protocol the typical yield from 500 g seeds was 5 g of quinoa extract (QE) containing 1.9% of 20E.

2.3. Measurement of energy expenditure components

The goal was to obtain for each mouse measurements of feeding pattern, spontaneous activity, total energy expenditure, respiratory quotient (RQ) and nutrient oxidation during 24 h ad libitum LFD and HFD feeding. Briefly, mice were randomly placed into 4 individual metabolic chambers equipped with a weighed food cup (sensitivity better than 0.05 g) and an activity platform (sensitivity better than 0.5 g). Since energy expenditure required for thermoregulation can be strongly increased when mice are singly housed at ambient temperature below 30 °C [23], we attempted to limit energy expenditure for thermoregulation by maintaining the temperature in the metabolic cages at 26–28°. After a 24-hour acclimation period, VO_2, VCO_2, feeding pattern and spontaneous motor activity were measured during 24 h.

For gas analysis a constant air flow (0.5 L/min), monitored by a mass-sensitive flow controller, was drawn through the chambers. The cages were multiplexed, i.e. connected in turn to the same gas analysers. VO_2 and VCO_2 were measured on each cage during 2 min every 10 min (2 min for each cage, plus 2 min on room air to correct VO_2 and VCO_2 values for room O_2% and CO_2%). Total energy expenditure (EE) was calculated from VO2 and VCO2 using Weir's formula