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Original Contribution

Fat intake reverses the beneficial effects of low caloric intake on skeletal muscle mitochondrial H_2O_2 production

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Abstract

Food restriction is the most effective modulator of oxidative stress and it is believed that a reduction in caloric intake per se is responsible for the reduced generation of reactive oxygen species (ROS) by mitochondria. Hydrogen peroxide (H_2O_2) generation and oxygen consumption (O_2) by skeletal muscle mitochondria were determined in a peculiar strain of rats (Lou/C) characterized by a self-low-caloric intake and a dietary preference for fat. These rats were fed either with a standard high-carbohydrate (HC) or a high-fat (HF) diet and the results were compared to those measured in Wistar rats fed a HC diet. H_2O_2 production was significantly reduced in Lou/C rats fed a HC diet; this effect was not due to a lower O_2 consumption but rather to a decrease in rotenone-sensitive NADH-ubiquinone oxidoreductase activity and increased expression of uncoupling proteins 2 and 3. The reduced H_2O_2 generation displayed by Lou/C rats was accompanied by a significant inhibition of permeability transition pore (PTP) opening. H_2O_2 production was restored and PTP inhibition was relieved when Lou/C rats were allowed to eat a HF diet, suggesting that the reduced oxidative stress provided by low caloric intake is lost when fat proportion in the diet is increased.

Keywords: Self-low-caloric intake; Free radicals; High-fat diet; Membrane potential; Uncoupling proteins; Calcium retention capacity

Introduction

In many species including mammals, reduction in energy intake without malnutrition is able to reduce the total amount of oxidative stress by altering mitochondrial metabolism, thereby delaying the onset of age-related pathologic conditions [1–3]. However, it has been shown that effects of low energy intake depend on different factors, such as the grade and the duration of energy restriction, the implementation time, and the tissue considered (see review in [3]). Thus, long-term reduction of caloric intake up to 1 year is generally accompanied by a decrease in mitochondrial H_2O_2 production and oxidative damages in rat heart and skeletal muscle [4–6] whereas in the liver reactive oxygen species (ROS) production was reported either to decrease [7] or not [8]. The effects of short-term caloric restriction (2 weeks to 4 months) on mitochondrial oxidative stress are even more controversial since H_2O_2 production by

Abbreviations: ROS, reactive oxygen species; HC, high carbohydrate; HF, high fat; ETC, electron transport chain; PTP, permeability transition pore; EE, energy expenditure; TEE, total EE; EEdark, EE during the dark phase; BSA, bovine serum albumin; BCA, bicinchoninic acid; $TPMP^+$, triphenylmethylphosphonium cation; RT-PCR, reverse-transcription polymerase chain reaction; T3, triiodothyronine; T4, tetraiodothyronine; Ca-RC, Ca^{2+} retention capacity; G/M, glutamate/malate; P/M, pyruvate/malate; CsA, cyclosporin A; CR, caloric restriction.

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skeletal muscle mitochondria has been reported to decrease in some case [9] or to remain unchanged [10]. Thus, with the available information it cannot be concluded whether skeletal muscle mitochondrial ROS production decreases or not with caloric restriction. Therefore, we considered of interest to investigate mitochondrial ROS production in a peculiar strain of rats (Lou/C) of Wistar origin [11] which is characterized by a self-low-caloric intake [12–16]. The most important difference between Lou/C and more common rat strains (Fisher 344, Wistar, Brown Norway) is a shift of preferences from carbohydrate to fat diets with aging [16] and the absence of obesity when fed a high-fat diet [15].

The major purpose of the present study was to measure mitochondrial ROS production in Lou/C rats fed with a standard diet rich in carbohydrates (HC) and the results were compared with Wistar rats fed the same diet and with Lou/C allowed to increase their fat intake. Since mitochondrial ROS generation was found to be dependent upon the electron flow through the electron transport chain (ETC), [17–19], H_2O_2 production was evaluated in skeletal muscle mitochondria energized with various substrates providing reducing equivalents to complex I or/and complex II of the ETC. For this purpose, skeletal muscle mitochondria were energized either with glutamate/malate (providing NADH) and/or succinate (generating $FADH_2$) as substrates. Finally, we examined the role of ROS on mitochondrial permeability transition that has been shown to be implicated in the control of apoptosis [20]. Indeed, the regulation of permeability transition pore (PTP) opening in skeletal muscle mitochondria is modulated by electron flow through complex I of the ETC [21] and the role of apoptosis in various tissues has been highlighted during normal aging [22] whereas Lou/C rats are suspected to display delayed aging [13].

Material and methods

Animals and experimental design

Four- to five-month-old male Lou/C and Wistar rats bred in our animal room facilities were caged individually and maintained at 22 ± 1°C, 12 h:12 h light: dark conditions, and 50 ± 10% relative humidity. Lou/C rats were divided into two groups, the first one (Lou/C-HC) being maintained on a standard high-carbohydrate (HC) rat chow (UAR AO3, Villemoisson, France) while the other group (Lou/C-HF) had the choice between the standard chow and a high-fat diet (HF, Table 1) which were both provided ad libitum in stainless-steel hanging cages. Body weight and food intake were recorded thrice weekly and fresh food was provided at the same time to ensure minimal disturbance to the animals' food behavior. Animals were cared for in accordance with guidelines from the French Ministère de l'Agriculture and the European Convention for the protection of Vertebrate Animals used for Experimental and Scientific purposes (Council of Europe No.123, Strasbourg, 1985).

Table 1
Composition of the diets

	Standard chow (UAR A03) g/100 g dry wt	High-fat diet g/100 g dry wt
Cornstarch	62	0
Casein	22.7	30
Vegetable oils	4.5	5
Lard	0	48
Mineral–vitamins	6.25	7.0
Cellulose	4.5	10.0
Energy value [kcal/100 g]	379	597

Diets were supplied by **U**sine **A**limentation **R**ationnelle (Epinay/Orge, France).

Whole body oxygen consumption–locomotor activity

Energy expenditure (EE) of individual Wistar and Lou/C-HC rats was monitored by indirect calorimetry as described previously [23]. Fractional concentrations of O_2 and CO_2 were measured using a paramagnetic analyzer (Servomex OA184) and an infrared gas analyzer (Servomex series 1400), respectively. Air flow rate was obtained from a Platon SDF 2044 flow meter. Data were collected online and processed with Labetech-Notebook software. Rats were placed individually in the respiration chamber early in the morning until the next mid-day so that the measurement lasted 24 h with at least 4 h of measurement before for rat habituation. Total EE (TEE) was calculated on an hourly basis and EE during the dark phase (EEdark) was calculated between 19:00 h and 07:00 h. During data collection, rats were fed ad libitum and kept on the same photoperiod and temperature as in the animal room facility.

Locomotor activity from Wistar and Lou/C-HC rats was measured with a Digiscan Animal Activity Monitor system (Omnitech Electronics Inc., Columbus, OH) which monitored the horizontal (back and forth; right and left) and vertical (rearing) movements of the animals. The Digiscan analyzer was interfaced with an IBM-PC-compatible computer using Digipro software. The individual compartments (L = 20; W = 20; H = 30 cm) were put in a dimly lit and quiet room. Horizontal, i.e., locomotion, and vertical movements, i.e., rearing were expressed as a number of beams crossed over 24 h of testing.

Sacrifice and tissue sampling

Rats were sacrificed by cervical dislocation for the collection of tissues and blood. Serum was extracted and flash-frozen in liquid nitrogen. Quadriceps from both legs were quickly excised, fat was eliminated, and the tissue was rinsed and chopped into isolation medium (150 mM sucrose, 75 mM KCl, 50 mM Tris-HCl, 1 mM KH_2PO_4, 5 mM $MgCl_2$, 1 mM EDTA, pH 7.4). Muscle pieces were transferred to 30 ml of isolation medium supplemented with