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Research paper

Early differences in metabolic flexibility between obesity-resistant and obesity-prone mice

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ABSTRACT

Decreased metabolic flexibility, i.e. a compromised ability to adjust fuel oxidation to fuel availability supports development of adverse consequences of obesity. The aims of this study were (i) to learn whether obesity-resistant A/J and obesity-prone C57BL/6J mice differ in their metabolic flexibility right after weaning; and (ii) to characterize possible differences in control of glucose homeostasis in these animals using glucose tolerance tests (GTT). A/J and C57BL/6J mice of both genders were maintained at 20 °C and weaned to standard low-fat diet at 30 days of age. During the first day after weaning, using several separate animal cohorts, (i) GTT was performed using 1 or 3 mg glucose/g body weight (BW), while glucose was administered either orally (OGTT) or intraperitoneally (IPGTT) at 20 °C; and (ii) indirect calorimetry (INCA) was performed, either in a combination with oral gavage of 1 or 7.5 mg glucose/g BW, or during a fasting/re-feeding transition. INCA was conducted either at 20 °C or 34 °C. Results of both OGTT and IPGTT using 1 mg glucose/g BW at 20 °C, and INCA using 7.5 mg glucose/g BW at 34 °C, indicated higher glucose tolerance and higher metabolic flexibility to glucose, respectively, and lower fasting glycemia in A/J mice as compared with C57BL/6J mice. Thus, control of whole body glucose metabolism between A/J and C57BL/6J mice represents a phenotypic feature differentiating between the strains right after weaning.

1. Introduction

Type 2 diabetes is often associated with obesity and related metabolic disorders. It reflects systemic metabolic changes resulting from excessive accumulation of body fat [1]. Thus, while lean and insulin-sensitive subjects are metabolically flexible, in obese and particularly in type 2 diabetic subjects the capacity to adapt fuel oxidation to fuel availability is compromised, reflecting in large the resistance of muscle metabolism to insulin [2]. Fatty acid oxidation in the fasted state is decreased as is the ability to efficiently switch to glucose oxidation in the post-prandial state [2]. However, some obese individuals remain metabolically healthy and even obese monozygotic twins can differ in their metabolic health [3]. Therefore, deterioration of metabolic flexibility represents one of the key self-standing markers of the development of type 2 diabetes, which could precede the outbreak of the disease by several years [4]. Metabolic flexibility is typically assessed using indirect calorimetry (**INCA**) as the response in respiratory quotient (**RQ**) to change in energy fuel [2]. In human studies, INCA is often used to assess metabolic flexibility either during an euglycemic-hyperinsulinemic clamp or during a meal test [2]. In laboratory

Abbreviations: AMPK, AMP-activated protein kinase; AU, arbitrary units; AUC, area under the curve; BW, body weight; B6, C57BL/6J mice; EE, energy expenditure; GTT, glucose tolerance test; HMW, high molecular weight; INCA, indirect calorimetry; IPGTT, intraperitoneal glucose tolerance test; LMW, low molecular weight; MMW, medium molecular weight; NEFA, non-esterified fatty acid(s); OGTT, oral glucose tolerance test; RQ, respiratory quotient; TAG, triacylglycerol(s); VO_2, oxygen consumption; VCO_2, carbon dioxide production.

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rodents, metabolic flexibility is typically evaluated using INCA during fasting/re-feeding transition [5,6] rather than in conjunction with the technically demanding clamp. Recently, an alternative approach was introduced in our group, to measure metabolic flexibility in mice as a response in RQ to a glucose bolus during INCA [7]. The information obtained using INCA could be complemented using glucose tolerance test (**GTT**), which monitors the homeostatic blood glucose clearance in response to a glucose challenge and reflects multiple mechanisms [8].

Inbred strains of mice are frequently used to characterize the mechanisms underlying development of obesity and associated metabolic disorders, with the obesity-resistant A/J mice and obesity-prone C57BL/6J (**B6**) mice [9,10], as two important models for the respective conditions. Body weight (**BW**) of mice of the A/J strain is lower than that of the B6 mice when fed standard low-fat diet and the A/J mice exhibit a relatively fast and strong induction of leptin by high-fat diet. In contrast, B6 mice increase their leptin levels only in association with increase in BW in response to high-fat diet, and the leptin levels in B6 mice exceed those in A/J only after B6 mice develop massive obesity [9,11]. Leptin supplementation of B6 mice does not prevent high-fat diet-induced obesity [12]. Thus, resistance of tissue metabolism to leptin, together with a relatively weak sensitivity to adrenergic stimulation [13], could contribute to the susceptibility of B6 to diet-induced obesity. It has also been shown that in adulthood and with free access to the standard diet, A/J mice exhibited (i) higher energy expenditure in the resting conditions reflecting liver β-oxidation, (ii) lower running endurance capacity reflecting muscle β-oxidation, and (iii) preferential activation of carbohydrate over lipid oxidative metabolism during exercise, as compared with B6 mice [14]. Whether all these differences contribute to the differential susceptibility to obesity remains to be clarified. In fact, the low obesogenic effect of high-fat diet in adult A/J mice has been ascribed to inducibility of liver lipid metabolism, including both mitochondria [15] and peroxisomes [16,17]. During a 2-week-period after weaning, whole-body lipid oxidation as well as muscle β-oxidation could be induced by high-fat diet in A/J mice but not in B6 mice, in association with a rescue of cold-tolerance of the A/J mice [6]. These results support the role of metabolic flexibility to lipids, the major fuel for non-shivering thermogenesis in brown fat and possibly also in other tissues, in the differential induction of non-shivering thermogenesis by high-fat diet in mice of the two strains early after weaning. However, possible differences in the control of carbohydrate metabolism between post-weaning mice of the two strains need to be further characterized.

To assess metabolic flexibility to glucose, mice of both A/J and B6 strains were studied right after weaning using different INCA protocols. These measurements were complemented by GTT. Using both approaches, profound differences in the control of glucose metabolism were found, with A/J mice showing relatively high metabolic flexibility and glucose tolerance.

2. Material and methods

2.1. Animals and diets

Experiments were performed using B6 and A/J mice of both genders (mice from the breeding colonies established at the Institute of Physiology in 1993 and 1997, respectively; both strains were imported from the Jackson Laboratories, Bar Harbor, ME). Mice were housed at 20 °C, with a 12-h light cycle, with a free access to water and breeding diet (Ssniff M-Z; proteins 36%, carbohydrates 53%, lipids 11% of energy content; 13.9 kJ/g). At 30 days of age, between 7:00 and 9:00 a.m., pups were removed from their breeding nests and they were single-caged, their BW was evaluated and they were offered pre-weighted standard low-fat diet (Ssniff R/M-H; proteins 33%, carbohydrates 58%, lipids 9% of energy content; 13 kJ/g; both diets from Ssniff Spezialdieten GmbH, Soest, Germany). At 6:00 a.m. the following day (i.e., one day after weaning), diet was removed, food intake was recorded and mice remained with free access to water, but without food, until GTT or INCA was performed (starting at 12:00 p.m.; except for INCA performed using a fasting/re-feeding protocol see below). All experiments were conducted in accordance with the guidelines for the use and care of laboratory animals of the Institute of Physiology (Approval Number: 172/2009), the directive of the European Communities Council (2010/63/EU), and the *Principles of Laboratory Animal Care* (NIH publication no. 85-23, revised 1985).

2.2. Glucose tolerance

At 12:00 a.m., after 6-h-fasting, GTT was performed at 20 °C, using either 1 mg or 3 mg glucose/g BW. Glucose was either injected intraperitonealy (**IPGTT**) using water solution of 10% D-glucose, wt/vol, or administered orally (**OGTT**) as intragastric gavage of water solution of 10% (1 mg glucose/g BW) or 50% (3 mg glucose/g BW) D-glucose, wt/vol, respectively. Tail blood was sampled at 0 (fasting blood glucose, just before glucose administration), 15, 30, 60, and 120 min; glycemia was measured using a glucometer (LifeScan, USA). To avoid the confounding effect of different baseline glucose levels, incremental rather than total AUC was evaluated. To reveal possible effects of mice handling, injection and gavage on glycemia, additional tests were conducted using 0.9% NaCl solution instead of glucose using separate groups of mice.

2.3. Energy metabolism

Two different INCA protols were applied while whole-body metabolic response to either intragastric glucose bolus or re-feeding the standard low-fat diet was characterized. In both protocols, animals were fasted for 5 h before glucose administration, or re-feeding, respectively.

INCA with a glucose bolus: INCA was conducted either at 20 °C or 34 °C after 3-h-fasting, for 2 h between 9:00 a.m. and 11:00 a.m. in a fasting state, then the measurement was interrupted and a bolus of either 1 mg or 7.5 mg glucose/g of BW was administered by intragastric gavage using water solution of 10% (1 mg glucose/g BW) or 50% (7.5 mg glucose/g BW) D-glucose, wt/vol, and the measurement continued for 4 h until 3:00 p.m.

INCA during fasting/re-feeding: INCA was conducted at 34 °C, starting at 1:00 p.m., when diet was removed. At 6:00 p.m, blood samples were collected using tail bleeding (fasted state) and pre-weighted ratio of the standard low-fat diet was administered, and INCA continued until 8:00 a.m. the following day when blood samples were collected again (re-fed state). Overnight food consumption was determined by weighting the diet remaining in the cage; no spillage of diet was detected.

INCA was performed similarly as before [6] using an 8-chamber system (Somedic, Horby, Sweden) and individually caged mice (Eurostandard type II mouse plastic cages; ~6000 ml; Techniplast, Milan, Italy). The cages were placed in sealed measuring chambers equipped with thermostatically controlled heat exchangers. Oxygen consumption (***VO*$_2$**; ml O_2/min) and carbon dioxide production (***VCO*$_2$**; ml CO_2/min) were recorded every 2 min under a constant airflow (1000 ml/min) in each chamber simultaneously. To assess fuel partitioning, RQ was calculated (RQ = VCO_2/VO_2). Energy expenditure (**EE**; cal) was calculated using the equation $3.9*VO_2$ (ml) + $1.1*VCO_2$ (ml) [18,19].