Original article

MOLECULAR METABOLISM

Adipose tissue (P)RR regulates insulin sensitivity, fat mass and body weight

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ABSTRACT

Objective: We previously demonstrated that the handle-region peptide, a prorenin/renin receptor [(P)RR] blocker, reduces body weight and fat mass and may improve insulin sensitivity in high-fat fed mice. We hypothesized that knocking out the adipose tissue (P)RR gene would prevent weight gain and insulin resistance.

Methods: An adipose tissue-specific (P)RR knockout (KO) mouse was created by Cre-loxP technology using AP2-Cre recombinase mice. Because the (P)RR gene is located on the X chromosome, hemizygous males were complete KO and had a more pronounced phenotype on a normal diet (ND) diet compared to heterozygous KO females. Therefore, we challenged the female mice with a high-fat diet (HFD) to uncover certain phenotypes. Mice were maintained on either diet for 9 weeks.

Results: KO mice had lower body weights compared to wild-types (WT). Only hemizygous male KO mice presented with lower total fat mass, higher total lean mass as well as smaller adipocytes compared to WT mice. Although food intake was similar between genotypes, locomotor activity during the active period was increased in both male and female KO mice. Interestingly, only male KO mice had increased O_2 consumption and CO_2 production during the entire 24-hour period, suggesting an increased basal metabolic rate. Although glycemia during a glucose tolerance test was similar, KO males as well as HFD-fed females had lower plasma insulin and C-peptide levels compared to WT mice, suggesting improved insulin sensitivity. Remarkably, all KO animals exhibited higher circulating adiponectin levels, suggesting that this phenotype can occur even in the absence of a significant reduction in adipose tissue weight, as observed in females and, thus, may be a specific effect related to the (P)RR.

Conclusions: (P)RR may be an important therapeutic target for the treatment of obesity and its associated complications such as type 2 diabetes.

Keywords (Pro)renin receptor; Renin-angiotensin system; Adipose tissue knock-out mice; Obesity; Adipose tissue; Insulin resistance

1. INTRODUCTION

Obesity and type 2 diabetes constitute worldwide pandemics [1,2]. Finding effective ways to prevent and treat these diseases is of major clinical and economic importance. The study of patients suffering from obesity and obese rodent models has shed light on the importance of the renin-angiotensin system (RAS) in the development of obesity [3,4]. In particular, adipose tissue RAS has been found to be increased in this condition, along with adipose tissue hypoxia and inflammation [5]. Those changes lead to compromised adipocyte function characterized by abnormal adipokine secretion and glucose and lipid metabolism, which together cause insulin resistance and increase the risk of developing type 2 diabetes [5]. Indeed, overexpression of angiotensinogen (Agt) in rodents, specifically in adipose tissue, causes obesity and insulin resistance, while systemic suppression of RAS genes protects from high-fat diet (HFD) induces obesity and improves insulin sensitivity [6]. In addition, clinical studies have shown that RAS inhibitors routinely used as anti-hypertensive treatments also improve insulin sensitivity and glucose homeostasis and prevent cardiovascular complications in diabetic patients [5].

The prorenin/renin receptor [(P)RR] has been shown to be a component of the RAS where its main role is to produce a non-proteolytic activation of prorenin and increase the catalytic activity of renin to cleave Agt into angiotensin I (Ang I) [7]. Ang I is then converted by the Ang converting enzyme to Ang II, the main physiologically active peptide of this system [8]. Ang II, via activation of many pathways, promotes cell

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Abbreviations: ANG, Angiotensin; BAT, brown adipose tissue; BB, beam break; HACT, horizontal activity; HFD, high-fat diet; HRP, handle-region peptide; KO, knock-out; ND, normal diet; OGTT, oral glucose tolerance test; PGF, perigonadal fat; PPAR-γ, peroxisome proliferator-activated receptor-γ; PRA, plasma renin activity; PRF, perirenal fat; (P)RR, prorenin/renin receptor; RAS, renin-angiotensin system; SE, standard error; SFC, abdominal subcutaneous fat; SM, skeletal muscle; SMG, submandibular gland; TG, triglycerides; V-ATPase, vacuolar proton pump H+-ATPase; VCO_2, carbon dioxide production; VO_2, oxygen consumption; WT, wild-type

Received August 3, 2016 • Revision received August 12, 2016 • Accepted August 16, 2016 • Available online xxx

http://dx.doi.org/10.1016/j.molmet.2016.08.009

proliferation, fibrosis, and apoptosis, which could lead to obesity-related complications, such as type 2 diabetes [9,10]. Simultaneously, binding of prorenin/renin to the (P)RR activates Ang II-independent pathways, including mitogen-activated protein kinases and tumor growth factor beta, which may also contributes to end-organ damage [11]. Unfortunately, the RAS inhibitors currently used clinically all cause a compensatory increase in plasma renin concentrations and, as a result, may increase (P)RR Ang II-independent pathways as these drugs do not prevent renin from binding to the (P)RR [12,13].

Independently of prorenin/renin binding, the (P)RR (also known as ATP6AP2) acts as a adapter to the cell membrane vacuolar proton pump H+-ATPase (V-ATPase), and consequently activates Wnt signaling [11]. Given that this signaling cascade is implicated in embryogenesis, whole-body suppression of the (P)RR is lethal [14,15]. Moreover, mice with specific (P)RR gene suppression in the heart or kidneys die at 2–4 weeks of age [16,17].

Our previous results showed that obese mice and insulin resistant obese women have increased adipose tissue (P)RR expression [18]. Furthermore, we demonstrated that systemic treatment of HFD-fed mice with the handle region peptide (HRP), a (P)RR blocker, reduces body weight gain and visceral fat masses and may improve insulin sensitivity [18], similarly to what is observed in models of RAS gene suppression [6]. The aim of the present study was to better understand the role of adipose tissue (P)RR in the metabolic effects induced by HRP treatment in the context of obesity using adipose tissue specific (P)RR KO mice.

2. MATERIALS AND METHODS

2.1. Generation of adipose-tissue specific (P)RR KO mice

To produce mice with the (P)RR gene deleted specifically in adipose tissue, mice expressing the Cre-recombinase specifically in adipose tissue under the control of the AP2 promoter (AP2-Cre^{Salk} mice; [19,20]; #005069, Jackson Lab) were bred with mice with loxP sites flanking the (P)RR exon 2 between locus 2271–2276 ((P)RR-Loxp mice; a generous gift from Merck Frosst Canada) (Supplemental Figure 1). As the (P)RR gene is present on the X chromosome [11], male mice were homozygous KOs while female mice were heterozygous KOs.

2.2. Animals

Mice were placed in individual cages at 10 weeks of age and were maintained on a normal diet (ND; #2918, Teklad Global, Harlan Laboratories, Madison, WI, USA) and 12-h light/dark cycle. Starting at 12 weeks of age, mice were maintained on the ND or switched to a HFD (Bio-Serv F3282, Frenchtown, NJ, USA) for 9 weeks. Mice had access to water and food *ad libitum*. Body weight and food intake were measured weekly from 12 to 17 weeks of age. Care of the mice used in these experiments complied with standards for the care and use of experimental animals set by the Canadian Council for the Protection of Animals, and all procedures were approved by the university's Animal Care and Use Committee at the CHUM Research Center.

2.3. Mouse genotyping

Mouse genotypes were determined using genomic DNA from mouse tail snips extracted by hot-shot NaOH-EDTA protocol [21,22]. PCR were then performed using specific primers (IDT IL, USA) detailed in Supplementary Table 1. Each reaction contained 1 μl 10× buffer, 0.2 μl 10 mM dNTP, 0.1 μl of each primer, 6.4 μl of water, 0.5 μl Taq polymerase (Feldan, Bio-Basic, Markham, ON, Canada), and 2 μl of genomic DNA, as described previously [23]. PCR products were subsequently analyzed on 1% or 3% agarose gels containing SYBR Green (Invitrogen by Life Technologies, Carlsbad, CA, USA).

2.4. Adipose tissue specificity of the KO model

The issue specificity of our KO model was confirmed by real-time PCR using the primers listed in Supplementary Table 1. For this purpose, 12 week-old male mice were sacrificed, and different white adipose tissue depots (abdominal subcutaneous fat (SCF) and visceral fat (perigonadal fat (PGF) and perirenal fat (PRF)) were collected, weighed, flash frozen, and analyzed by real-time PCR. (P)RR gene expression was assessed in the different white adipose tissue depots as well as in other tissues such as, brown adipose tissue (BAT), heart, spleen, gonads, kidneys, submandibular gland, pancreas, liver, brain, adrenal glands, skeletal muscle, lung, diaphragm, and aorta.

2.5. Reverse transcription and qPCR gene expression

Tissue RNA was extracted using Trizol reagent (Invitrogen, Burlington, ON, Canada) according to the manufacturer's protocol. To remove genomic DNA, RNA samples were incubated with deoxyribonuclease I (2U per μg RNA) for 30 min at 37 °C. Single-stranded cDNA was synthesized by reverse-transcriptase reaction using SuperScript II Reverse Transcriptase (FisherScientific, Ottawa, ON, Canada). The RT-PCR final volume was 10 μl and contained 0.3 μmol/l of the specific forward (F) and reverse (R) primers [24] listed in Supplementary Table 1, as well as 2.5 μl of single-stranded cDNA template. The amplification was done using Faststart SYBR Green Master fluorescent dye (04 673 492 001; ROCHE, Mississauga, ON, Canada) using the Rotor Gene RG-3000 (Corbett Research).

2.6. Survival rate and fetal weight

We compared fetal weights in litters obtained from 8 pregnant mice (3–11 pups/litter), produced using the breeding protocol mentioned above, on the 17th day of gestation.

2.7. Body composition

Fat and lean body masses were analyzed using an Echo-MRI-100TM apparatus (Echo Medical Systems, Houston Scientific, Houston, TX, USA) both at the beginning and at the end of the protocol (at 12 and 20 weeks of age, respectively).

2.8. Mouse locomotor activity and indirect calorimetry

Mouse locomotor activity and metabolic parameters were studied in metabolic cages at 18 weeks of age (AccuScan Instruments, Columbus, OH, USA), and data were analyzed according to Ferrannini et al., 1988 [25]. After 3 days of acclimation, locomotor activity was evaluated by infrared beam interruptions by mouse movement in the horizontal and vertical axis, while metabolic parameters were assessed (oxygen consumption (VO_2) and carbon dioxide production (vCO_2)) for 24 h. Data were summarized for both the light and dark cycles [26].

2.9. Oral glucose tolerance test (OGTT)

At 20 weeks of age, an OGTT was performed after overnight (16–17 h) fasting. Dextrose (Hospira, Inc. Lake Forest, Il, USA) was administered orally by gavage at a dose of 2 g/kg for ND-fed mice or 5 g/kg for HFD-fed mice [27–29]. Blood was collected from the tail vein at baseline (T0) and 15, 30, 45, 60, and 120 min after gavage using a glucometer (Accu-Chek Performa, Roche, Indianapolis, IN, USA). Plasma was also collected at baseline (T0) and 30 min after gavage using glass capillaries (Precision, MO, USA), then transferred into BD vacutainer tube and separated by centrifugation (Becton, Dickenson, Mississauga, ON,

