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ACE2 deficiency shifts energy metabolism towards glucose utilization

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ABSTRACT

Background. This study aimed at investigating the effects of genetic angiotensin-converting enzyme (ACE) 2 deficiency on glucose homeostasis in the pancreas and skeletal muscle and their reversibility following ACE inhibition.

Procedures. ACE2-knockout and C57bl6J mice were placed on a standard diet (SD) or a high-fat diet (HFD) for 12 weeks. An additional group of ACE2-knockout mice was fed a SD and treated with the ACE inhibitor, perindopril (2 mg kg^{-1} day^{-1}). Glucose and insulin tolerance tests, indirect calorimetry measurements and EchoMRI were performed. Non-esterfied 'free' fatty acid oxidation rate in skeletal muscle was calculated by measuring the palmitate oxidation rate. β-cell mass was determined by immunostaining. Insulin, collectrin, glucose transporter protein, and peroxisome proliferator-activated receptor-γ expression were analysed by RT-PCR. Markers of mithocondrial biogenesis/content were also evaluated.

Main Findings. ACE2-knockout mice showed a β-cell defect associated with low insulin and collectrin levels and reduced compensatory hypertrophy in response to a HFD, which were not reversed by perindopril. On the other hand, ACE2 deficiency shifted energy metabolism towards glucose utilization, as it increased the respiratory exchange ratio, reduced palmitate oxidation and PCG-1α expression in the skeletal muscle, where it up-regulated glucose transport proteins. Treatment of ACE2-knockout mice with perindopril reversed the skeletal muscle changes, suggesting that these were dependent on Angiotensin II (Ang II).

Abbreviations: ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; Ang, angiotensin; AT receptor, angiotensin II receptor; GLUT-1, glucose transporter-1; GLUT-4, glucose transporter-4; HFD, high-fat diet; IGF-1, insulin growth factor-1; IPGTT, intraperitoneal glucose tolerance test; IPITT, intraperitoneal insulin tolerance test; NEFA, non-esterified 'free' fatty acid; NRF-1, nuclear respiratory factor-1; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1 alpha; PPARγ, peroxisome proliferator-activated receptor-γ; RAAS, renin-angiotensin-aldosterone system; RER, respiratory exchange ratio; SD, standard diet; T2DM, type 2 diabetes mellitus; ZDF, Zucker diabetic fatty.

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Principal Conclusions. *ACE2*-knockout mice display a β-cell defect, which does not seem to be dependent on Ang II but may reflect the collectrin-like action of ACE2. This defect seemed to be compensated by the fact that *ACE2*-knockout mice shifted their energy consumption towards glucose utilisation via Ang II.

1. Introduction

The renin-angiotensin-aldosterone system (RAAS) is not only a key homeostatic regulator of vascular function, but it also appears to have a role in metabolic homeostasis and diabetes development [1]. In type 2 diabetes mellitus (T2DM) experimental models, such as the ZDF rats, RAAS expression and activity are in fact up-regulated in the pancreatic islets, where RAAS blockade is able to attenuate islet damage and improve glucose tolerance [2].

The primary effector of the RAAS is the octopeptide Angiotensin (Ang) II, whose tissue and circulating levels are influenced by its rate of synthesis and degradation [3]. Angiotensin-converting enzyme (ACE) is the major enzyme that synthesizes Ang II, while ACE2 is the major enzyme that degrades Ang II [3]. Consequently, deletion or inhibition of ACE2 results in increased circulating and tissue levels of Ang II [3,4].

ACE2 is highly expressed in the pancreas, in both acini and islets [5]. Recent studies have shown that ACE2 deficiency is associated with a progressive impairment of glucose tolerance, possibly due to a selective decrease in first-phase insulin secretion in response to glucose [6]. This is consistent with the finding that adenovirus-mediated overexpression of ACE2 in the pancreas of *db/db* mice improves glycemic control by increasing islet cell insulin content [7]. Nevertheless, since ACE2 is expressed in the liver, adipose tissue, and skeletal muscle [8], it could also influence peripheral glucose utilization and insulin sensitivity, although these actions are less well understood [9].

Here we investigated the effects of ACE2 deficiency on glucose homeostasis by studying *ACE2*-knockout mice metabolic response to either a standard or a high-fat diet.

2. Material and methods

2.1. Animals and experimental protocol

A total of 18 adult (8-wk-old) male C57bl6J mice were randomly fed either a standard chow diet (C57 SD, n = 9) or a high-fat diet (C57 HFD, n = 9), together with 18 adult male *ACE2*-knockout mice (on a C57Bl6J background), which were also randomly fed either a SD (*ACE2*KO SD, n = 9) or a HFD (*ACE2*KO HFD, n = 9). The SD consisted of 19.6% of protein, 4.6% of total fat and 4.5% of crude fibre providing a digestible energy of 14.3 MJ/Kg. The HFD consisted of 22.6% of protein, 23.5% of total fat and 5.4% of crude fibre, providing a digestible energy of 19 MJ/Kg, and was formulated to mimic a "Western" fast-food diet [10] (SF04-001 diet or high fat rodent diet based on D12451, provided by Speciality Feeds, Glen Forrest Western Australia 6071). In addition, to evaluate whether the changes observed in *ACE2*KO mice were due to Ang II, 9 adult (8-wk-old) male *ACE2*KO mice were fed a standard chow diet and were treated with the ACE inhibitor perindopril (2 mg kg^{-1} day^{-1}) (*ACE2*KO SD + peri, n = 9).

All the animals were followed up for 12 weeks, and they were kept in a temperature-controlled room (22 ± 1 °C) on a 12-hour light/dark cycle with free access to food and water for the entire study duration. Measurements by indirect calorimetry were performed at week 11, intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT) were performed at week 12 when body composition and food intake were also recorded.

At the end of the study, animals were anesthetized by an intraperitoneal injection of pentobarbitone at a dose of 100 mg/kg of body weight. Blood was collected from the left ventricle and centrifuged, and plasma was stored at −20 °C for analysis. Skeletal muscles were used for *ex vivo* non-esterified 'free' fatty acid (NEFA) oxidation and RNA extraction, and immunostainings. Pancreases were also collected for RNA extraction and immunostainings. Principles of laboratory animal care were followed as well as specific national laws where applicable. This study was approved by the AMREP Animal Ethic Committee, the numbers of approval were 0369 and 0947, and the study period was May 2009–May 2012.

2.2. Metabolic parameters and body composition

Food intake was measured by placing the mice in metabolic cages for 24 hours. Total body mass, fat, and lean mass were evaluated by EchoMRI (Echo Medical Systems, Houston, TX).

2.3. Measurement of glucose, insulin and insulin growth factor-1

Glucose was measured using an automatic glucometer (AccuCheck II; Roche) during the IPGTT and the IPITT, and at the end of the study at fasting. Insulin was measured by ELISA (Cat no. EZRMI-13 K; Millipore) in the sera collected during the IPGTT, and at the end of the study at fasting. IGF-1 levels were measured by ELISA (Cat no. ac-18f1; Novozymes, IDS) in the plasma collected at the end of the study at fasting.

The IPGTT was performed on day 1 of week 12 by injecting glucose (2 g/kg) intraperitoneally after an overnight fast and then collecting blood at baseline and at 15, 60, and 120 min. Bloods were then centrifuged to collect the sera. The IPITT was performed on day 4 of week 12 by injecting insulin (1 unit/kg) intraperitoneally after a 6-hour fast and collecting bloods at baseline, 15, 60, and 120 min.

2.4. β-Cell mass assessment and other immunostainings

β-Cell mass assessment was performed by insulin immunostaining on 4 μm paraffin sections using a polyclonal mouse