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Adiponectin Receptor-1 Expression Is Decreased in the Pancreas of Obese Mice[1]

Terence E. Wade, M.D., Abhishek Mathur, M.D., Debao Lu, M.D., Deborah A. Swartz-Basile, Ph.D., Henry A. Pitt, M.D., and Nicholas J. Zyromski, M.D.[2]

Department of Surgery, Indiana University School of Medicine, Indianapolis, Indiana

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Background. **Obesity is epidemic in the 21st century and has been shown to be a risk factor for developing severe acute pancreatitis. Adipose tissue produces small molecules called adipokines, which are important in modulating metabolism and inflammation. The anti-inflammatory adipokine adiponectin is decreased in obesity and inversely mirrors the severity of pancreatitis in a murine experimental model. Adiponectin acts through two receptors, AdipoR1 and AdipoR2; no data are currently available regarding adiponection receptor expression in the obese murine pancreas.**

Materials and methods. **Immunohistochemical and reverse transcription-polymerase chain reaction analysis were undertaken to determine expression of adiponectin receptors AdipoR1 and AdipoR2 in the pancreas and liver of lean (C57BL/6J) and congenitally obese (Lep^{Ob} and Lep^{Db}) mice.**

Results. **Immunohistochemistry confirmed expression of both AdipoR1 and AdipoR2 in the pancreas of all three murine strains. Staining was positive in acinar cells and to a lesser extent in islet cells. Pancreatic gene expression of AdipoR2 was similar among lean and obese mice. AdipoR1 gene expression, however, was significantly ($P < 0.001$) decreased in the pancreas of both Lep^{Ob} and Lep^{Db} mice compared to wild-type lean animals. Gene expression of both AdipoR1 and AdipoR2 was significantly less in the liver of obese (Lep^{Ob} and Lep^{Db}) mice compared to wild-type lean animals ($P < 0.001$).**

Conclusions. **These data show for the first time that the adiponectin receptors AdipoR1 and AdipoR2 are expressed in the obese murine pancreas. The paucity of AdipoR1 receptors may be important when considering the role played by adipokines in the genesis of severe pancreatitis in obesity.**

Key Words: **adiponectin; adiponectin receptors; pancreatitis; obesity.**

INTRODUCTION

Obesity is epidemic in the 21st century. This burdensome problem affects over one-third of American adults and results in more than $120 billion in yearly health care costs [1]. Obesity leads to fatty infiltration and organ dysfunction in the heart, kidney, and liver [2–4]; recent evidence supports the concept that a similar situation occurs in the pancreas [5, 6]. The increased incidence of obesity has been accompanied by a renewed interest in adipose tissue biology, and the mechanisms by which fat contributes to the pathophysiology of disease are slowly being elucidated.

Adipose tissue is no longer viewed as an inert metabolic storage tissue. In fact, it is now recognized to be an important endocrine organ producing biologically active compounds collectively referred to as adipokines [7]. Adipokines are pleiotropic molecules that modulate metabolism and inflammation. Two of the most prominent, and therefore best, characterized adipokines are leptin and adiponectin. Leptin is integral in control of satiety and has also been shown to function as a proinflammatory agent in many systems [8]. Circulating leptin concentration increases in proportion to increased adipose tissue volume. Adiponectin, in contrast, is generally felt to be an anti-inflammatory adipokine. As obesity progresses, circulating adiponectin concentration paradoxically decreases [9, 10]. These changes in the adipokine milieu contribute to the generalized proinflammatory state observed in obesity.

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[2] To whom correspondence and reprint requests should be addressed at Department of Surgery, Indiana University School of Medicine, 535 Barnhill Drive, RT 130, Indianapolis, IN 46202. E-mail: nzyromsk@iupui.edu.

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Acute pancreatitis also represents a significant health care problem. Each year in the United States, over 240,000 patients are hospitalized with the primary diagnosis of acute pancreatitis, at a cost of over $2.3 billion [11]. It is generally accepted that premature enzyme activation is responsible for the initiation of the inflammatory response seen in pancreatitis; however, the exact pathophysiology is incompletely understood. The spectrum of illness in pancreatitis is broad, with clinical manifestations ranging from mild epigastric tenderness and pancreatic edema to severe, life-threatening destruction of pancreatic and peripancreatic tissue. The majority of patients with acute pancreatitis manifest a mild, self-limiting disease course. Approximately 15–20% of patients, however, develop severe acute pancreatitis with a massive systemic inflammatory response and local complications leading to distant organ failure, and mortality rates that reach 10–20% [12, 13]. Despite a tremendous amount of basic and clinical research, no specific therapeutic modality currently exists, and the treatment of acute pancreatitis remains entirely supportive. Clinical studies have consistently shown obesity to be an independent risk factor for developing severe pancreatitis [14–16]. Surprisingly little basic investigation has addressed this association, and the mechanisms underlying the lethal combination of obesity and pancreatitis remain completely unknown.

We have recently shown that similar to the human situation, congenitally obese mice develop more severe pancreatitis than wild-type lean animals [5]. In these experiments, the severity of pancreatitis was not solely related to volume of adipose tissue or concentration of the proinflammatory adipokine leptin. An intriguing observation, though, was that serum adiponectin concentration inversely mirrored the severity of pancreatitis, suggesting a protective effect provided by this anti-inflammatory adipokine. Two adiponectin receptors—AdipoR1 and AdipoR2—were identified in 2003 [17]. Current evidence suggests that AdipoR1 is expressed strongly in skeletal muscle and also distributed throughout other tissues including the gut. AdipoR2 seems to be limited more preferentially to the liver in most systems [17–19]. A more complete understanding of adiponectin physiology is in evolution, and no data are currently available regarding the expression of adiponectin receptors in the obese murine pancreas. Therefore, the aim of the current study was to define the presence and relative density of AdipoR1 and AdipoR2 in the pancreas of lean and congenitally obese mice.

MATERIALS AND METHODS

All studies were performed with approval of the Indiana University Institutional Animal Care and Use Committee and were in accordance with the National Research Council guide for the care and use of laboratory animals.

Animals and Diets

Sixteen lean (C57BL/6J), 8 obese leptin-deficient (Lep^{Ob}) and 8 obese leptin-resistant (Lep^{Db}) female mice were obtained from The Jackson Laboratory (Bar Harbor, ME) at 7 weeks of age. Mice were housed in a light- (12 h light:dark) and temperature- (22°C) controlled room. During 1 week of environmental adjustment, mice were fed a standard low fat chow diet (Ralston Purina, St. Louis, MO). At 8 weeks of age mice were fed a diet containing 25% fat (soybean oil + corn oil), 55% carbohydrate (sucrose and cornstarch), and 20% protein derived calories (Dyets Inc., Bethlehem, PA) *ad libitum* for a total of 4 weeks. Our lab has extensive experience and baseline data under these conditions. Animals and food were weighed weekly to monitor growth and dietary intake.

Tissue Collection

At 12 weeks of age, mice were then sedated with isoflurane and anesthetized with an intraperitoneal injection of xylazine (15 mg/kg) and ketamine (50 mg/kg). The animals then underwent laparotomy and total pancreatectomy. A portion of each pancreas was preserved in formalin for histological analysis and the remainder of each pancreas was snap frozen in liquid nitrogen and stored at −80°C for subsequent reverse transcription-polymerase chain reaction (RT-PCR) analysis. Liver and skeletal muscle were rapidly harvested and a portion of each was preserved in formalin and liquid nitrogen. Blood was collected by ventricular puncture and centrifuged at 15,000 rpm for 5 min to separate serum. Animals were then euthanized by exsanguination.

Immunohistochemical Analysis

Anti-AdipoR1 and anti-AdipoR2 human antibodies were obtained from Vector Laboratories (Burlingame, CA). Briefly, pancreatic specimens fixed in formalin, embedded in paraffin, and sectioned into 5-μm sections were rehydrated, and endogenous peroxidase activity was blocked with 3% H_2O_2 (Sigma Aldrich, St. Louis, MO). Slides were then blocked with 2% serum (Vector) and incubated overnight with 100 μL of primary antibody (0.5–5.0 μg/mL in Wash Buffer-Saponin, 1:100) at room temperature in a humidified chamber. Slides were then washed three times in PBS and subsequently incubated at room temperature for 30 min with Vectastain Elite ABC-peroxidase reagent (Vector), peroxidase, and substrate. Counterstaining was performed with hematoxylin. Murine liver and skeletal muscle were used as positive controls; negative control slides were stained without incubation with primary antibody. An independent and blinded observer reviewed each slide.

RT-PCR Analysis

To determine AdipoR1 and AdipoR2 mRNA expression levels in the murine pancreata, we used PCR technique. Pancreata from lean mice were pooled two per pool and pancreata from obese mice were pooled one per group. Total RNA was isolated using RNeasy (Qiagen, Valencia, CA) according to the manufacturer's instructions. Determination of concentration, quality, and integrity of total RNA was accomplished with an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA). First strand cDNA synthesis was performed with Super Script III Platinum Two-Step qRT-PCR kit (Invitrogen Life Technologies, Inc., Carlsbad, CA). The cDNA used in each reaction was derived from 100 ng of total RNA. Real-time PCR reactions were carried out on an ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA) using Platinum Quantitative PCR SuperMix-UDG according to the manufacturer's recommendation. The following specific primers were used: *Adiponectin Receptor 1 sense*: ACGTTGGAGAGTCATCCCGTAT; *Adiponectin Receptor 1 antisense*: CTCTGTGTGGATGCGGAAGAT; *Adiponectin Receptor 2 sense*: TCCCAGGAAGATGAAGGGTTTAT; *Adiponectin Receptor 2 antisense*: TTCCATTCGTTCGATAGCATGA (Invitrogen); ^{18}S rRNA was used as an internal control.