HPB, 2007; 9: 312–318

informa
healthcare

ORIGINAL ARTICLE

Nonalcoholic fatty pancreas disease

ABHISHEK MATHUR, MEGAN MARINE, DEBAO LU, DEBORAH A. SWARTZ-BASILE, ROMIL SAXENA, NICHOLAS J. ZYROMSKI & HENRY A. PITT

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

Abstract

Background. Obesity leads to fat infiltration of multiple organs including the heart, kidneys, and liver. Under conditions of oxidative stress, fat-derived cytokines are released locally and result in an inflammatory process and organ dysfunction. In the liver, fat infiltration has been termed nonalcoholic fatty liver disease, which may lead to nonalcoholic steatohepatitis. No data are available, however, on the influence of obesity on pancreatic fat and cytokines, and nonalcoholic fatty pancreas disease (NAFPD) has not been described. Therefore, we designed a study to determine whether obesity is associated with increased pancreatic fat and cytokines. *Materials and methods.* Thirty C57BL/6J lean control and 30 leptin-deficient obese female mice were fed a 15% fat diet for 4 weeks. At 12 weeks of age all animals underwent total pancreatectomy. Pancreata from each strain were pooled for measurement of a) wet and dry weight, b) histologic presence of fat, c) triglycerides, free fatty acids (FFAs), cholesterol, phospholipids, and total fat, and d) interleukin (IL)-1β and tumor necrosis factor-alpha (TNF-α). Data were analyzed by Student's *t* test and Fisher's exact test. *Results.* Pancreata from obese mice were heavier ($p < 0.05$) and had more fat histologically ($p < 0.05$). Pancreata from obese mice had more triglycerides, FFAs, cholesterol, and total fat ($p < 0.05$). Triglycerides represented 11% of pancreatic fat in lean mice compared with 67% of pancreatic fat in obese mice ($p < 0.01$). Cytokines IL-1β and TNF-α also were elevated in the pancreata of obese mice ($p < 0.05$). *Conclusions.* These data suggest that obese mice have 1) heavier pancreata, 2) more pancreatic fat, especially triglycerides and FFAs, and 3) increased cytokines. We conclude that obesity leads to nonalcoholic fatty pancreatic disease.

Key Words: *Cytokines, fat, obesity, pancreas*

Introduction

Obesity has become epidemic in the United States, with more than 50 000 000 Americans having a body mass index (BMI) >30 [1]. Obesity leads to multiple comorbidities including diabetes, hypertension, and hyperlipidemia (the metabolic syndrome). In addition, obesity causes fat infiltration of several organs including the heart, kidneys, and liver. Under conditions of oxidative stress, fat-derived cytokines are released locally and result in an inflammatory process and organ dysfunction. In the liver, fat infiltration has been termed nonalcoholic fatty liver disease (NAFLD), which may lead to nonalcoholic steatohepatitis (NASH) [2,3]. In addition, adipose tissue has been characterized as an endocrine organ with increased production of adipokines, including leptin and adiponectin, and cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and monocyte chemotactic protein-1 (MCP-1) [4]. Macrophages, in turn, produce IL-1β and myeloperoxidase (MPO), which further exacerbate the inflammatory process [4,5].

In 1920, Schaefer reported a correlation between the weight of the adult pancreas and body weight [6]. In 1933, Ogilvie found 9% pancreatic fat in lean cadavers compared with 17% pancreatic fat in obese cadavers [7]. In the 1960s and 1970s fat in the pancreas (pancreatic lipomatosis) was correlated with age, obesity, and type 2 diabetes [8,9]. Recent computed tomography (CT) and magnetic resonance imaging (MRI) studies also have correlated pancreatic fat with obesity [10–12]. Human observations further suggest that the severity of pancreatitis is increased in obese patients [13,14]. Despite these observations, nonalcoholic fatty pancreatic disease (NAFPD) and nonalcoholic steatopancreatitis (NASP) have not been described.

Correspondence: Henry A. Pitt, MD, Department of Surgery, Indiana University School of Medicine, 535 Barnhill Drive, RT 130D, Indianapolis, IN 46202, USA. Tel: +1 317 274 2304. Fax: +1 317 274 4554. E-mail: hapitt@iupui.edu

Presented at the International Hepato-Pancreato-Biliary Association, September 3–7, 2006, Edinburgh, UK.

(Received 5 February 2007; accepted 10 May 2007)

ISSN 1365-182X print/ISSN 1477-2574 online
DOI: 10.1080/13651820701504157

Materials and methods

Animals and diets

Thirty lean control (C57BL/6J) and 30 obese leptin-deficient (Lep^{ob}) female mice were obtained from Jackson Laboratory (Bar Harbor, ME, USA). Lep^{ob} mice are known to have islet cell hyperplasia, type II diabetes, and elevated serum glucose and insulin, suggesting pancreatic endocrine insufficiency [15,16]. The mice were housed five per cage in a light (6 am to 6 pm) and temperature (22°C) controlled room. During 1 week of environmental adjustment, the mice were fed a standard low fat chow diet (Ralston Purina, St Louis, MO, USA). At 8 weeks of age all the lean C57BL/6J and the obese leptin-deficient (Lep^{ob}) female mice were fed a low fat diet (15% fat, 45% carbohydrate, and 40% protein) (Dyets Inc., Bethlehem, PA, USA) for 4 weeks. The fat was anhydrous milk fat; the carbohydrates were 35% sucrose and 10% cornstarch; and the protein was casein. Both the animals and the food were weighed weekly to determine growth and dietary intake. All protocols for these animal studies were approved by the Indiana University Institutional Animal Care and Use Committee.

Serum and tissue collection

At 12 weeks of age, after an overnight fast with water allowed *ad libitum*, the mice were sedated with an isoflurane-soaked gauze placed in a 2000 cm^3 glass jar. They were then anesthetized with an intraperitoneal injection of xylazine (15 mg/kg) and ketamine (50 mg/kg). The animals were weighed and then underwent laparotomy and total pancreatectomy. A slice of tissue from eight dorsal pancreata from each strain was placed in formalin for measurement of histologic presence of fat, inflammation, and fibrosis. Seven pancreata from each strain were employed for measurement of wet and dry weights. Sixteen dorsal pancreata from each strain were pooled two per group and snap frozen to −80°C for measurement of triglycerides, free fatty acids (FFAs), cholesterol, phospholipids, and total fat. Seven dorsal pancreata from each strain were snap frozen to −80°C for measuring cytokines IL-1β and TNF-α. Whole blood was aspirated from the heart and centrifuged to isolate serum.

Histologic analysis

Pancreatic specimens fixed in formalin were stained with hematoxylin and eosin and were reviewed by an observer who was blinded as to the groups. Each specimen was graded, in five high powered fields, 0 to 4+ for inter- and intralobular fat, inflammation, and fibrosis. The total pancreatic fat score was calculated as a sum of the inter- and intralobular fat. Figure 1A, B shows a typical pancreas from a lean and an obese mouse, respectively.

Serum analysis

Whole blood was spun at 15,000 rpm for 5 min to separate serum. Serum was pooled to give six pools for lean mice, and five pools were obtained for obese mice. Serum cholesterol and triglycerides were determined by using an enzymatic colorimetric method for their quantitative determination. The kits for these measurements were obtained from Wako Chemicals USA, Inc., Richmond, VA, USA and Stanbio Labs, Boerne, TX, USA.

Gallbladder lipid analysis

Gallbladder lipids from eight pools from lean and obese mice underwent lipid analysis at the Mouse Metabolic Phenotyping Center at Vanderbilt University Medical Center, as previously described by Goldblatt et al. [17]. Briefly, lipids were extracted by

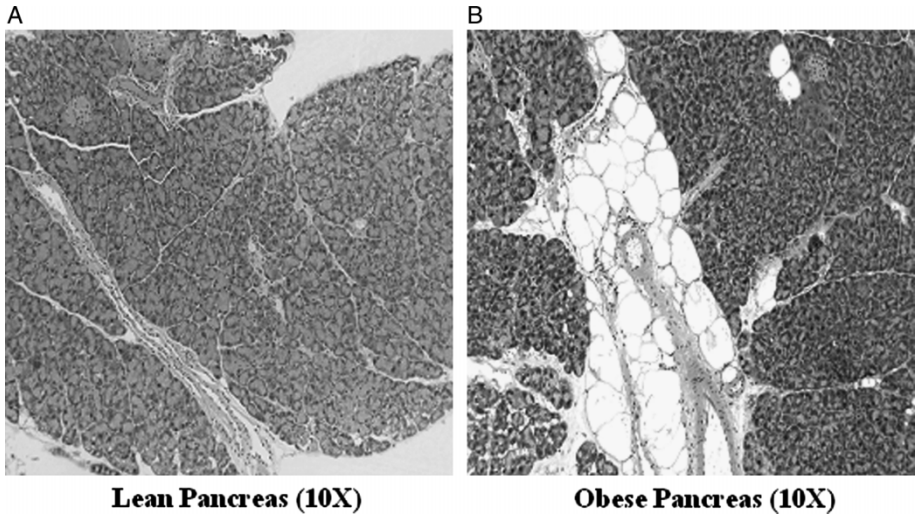

Figure 1. (A) Typical pancreatic histology (A) of a lean mouse and (B) of an obese mouse (original magnification ×10).