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Original Article

Metabolic Damage Presents Differently in Young and Early-Aged C57BL/6 Mice Fed a High-Fat Diet☆

Chia-Yuan Liu [1, 4, 6, 7 †], Ching-Wei Chang [1, 4, 6, 7 †], Hung-Chang Lee [2, 6, 7, 9 ‡], Yu-Jen Chen [3], Tung-Hu Tsai [4], Jen-Shiu Chiang Chiau [5], Tsang-En Wang [1, 6, 7], Ming-Chieh Tsai [8], Chun-Yan Yeung [2, 6, 7], Shou-Chuan Shih [1, 6, 7 * ‡]

[1] *Division of Gastroenterology, Department of Internal Medicine, MacKay Memorial Hospital,* [2] *MacKay Children's Hospital,* [3] *Department of Radiation Oncology, MacKay Memorial Hospital,* [4] *Institute of Traditional Medicine, National Yang-Ming University,* [5] *Department of Medical Research, Hsinchu MacKay Memorial Hospital, Hsinchu,* [6] *Department of Medicine, MacKay Medical College,* [7] *Mackay Junior College of Medicine, Nursing, and Management, New Taipei City,* [8] *Endocrinology and Metabolism, Department of Internal Medicine, MacKay Memorial Hospital,* [9] *Department of Pediatrics, Hsinchu MacKay Memorial Hospital, Hsinchu, Taiwan*

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SUMMARY

Background: Obesity in old individuals is increasing at alarming rates, and this population is more vulnerable to the deleterious metabolic effects of obesity than younger individuals. However, at present, there is no ideal obesity model to evaluate the interaction of aging and obesity.
Methods: The development of a metabolic damage model in response to a fixed period of high-fat diet (HFD) feeding was examined in mice of different ages. Mice aged 6 weeks (young group) and 44 weeks (elderly group) were fed a standard diet or HFD for 12 weeks, and their metabolic characteristics were studied. Inflammation was determined by the serum lipopolysaccharide (LPS) level. Gut microbiota composition was analyzed from stool samples.
Results: After 12-week feeding, weight gain; elevated serum levels of cholesterol, triglyceride, and LPS; increased homeostasis model assessment-estimated insulin resistance (HOMA-IR); fat accumulation; nonalcoholic fatty liver disease (NAFLD) activity score (NAS); and gut microbiota changes occurred in response to HFD feeding in both young and elderly groups ($p < 0.05$). HFD-induced serum levels of alanine aminotransferase, cholesterol, triglyceride, and insulin; HOMA-IR; fat accumulation; NAS; and gut microbiota changes were significantly higher in the elderly group ($p < 0.05$).
Conclusion: Aging in an obese mouse model does not increase LPS but exacerbates NAFLD severity, dyslipidemia, insulin insensitivity, fat accumulation, and gut microbiota changes. This obesity model might help elucidate target or multiple organ alterations associated with metabolic disorder and aging.

1. Introduction

A global epidemic of obesity is expected to be the leading cause of morbidity and mortality in the current and future generations. Obesity is a complex disorder involving excessive body fat. The metabolic consequences of obesity drive life-threatening disorders, including a spectrum of liver abnormalities such as nonalcoholic fatty liver disease (NAFLD) and steatohepatitis and serious cardiometabolic abnormalities, including Type 2 diabetes mellitus, dyslipidemia, metabolic syndrome, hypertension, atherogenesis, and coronary heart disease[1,2].

By 2030–2035, 20% of American adults and 25% of European adults will be 65 years or above[3]. The prevalence of obesity is rising progressively among the older age groups as well. In the United States, the prevalence of obesity in elderly Americans aged 60 years or above increased from 23.6% in 1990 to 32.0% in 2000 and 37.4% in 2010. In Taiwan, a study on participants aged above 65 years in

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* Correspondence to: Shou-Chuan Shih, Division of Gastroenterology, Department of Internal Medicine, Mackay Memorial Hospital, No. 92, Sec. 2, Chung-Shan North Road, Taipei, Taiwan. Tel.: +886 2 25433535x2004; fax: +886 2 25433642.
E-mail address: sschuan@mmh.org.tw (S.-C. Shih).
† These two authors contribute equally to this work.
‡ These two authors contribute equally to this work.

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2009 indicated that about 30% were overweight and 20% were obese[4]. Obesity in the elderly is thus a matter of serious concern.

The aging process is altered or accelerated when cardiometabolic diseases are present, and aging makes organisms more susceptible to such illnesses. Both obesity and aging are characterized by a low-grade inflammatory state and endocrine changes[3]. Central and visceral obesities are more proinflammatory than global obesity in elderly humans. The low-grade inflammatory state is associated with decreased lean body mass, reduced immune function, cognitive decline, insulin resistance, and several correlates of metabolic control and insulin resistance such as increased levels of tumor necrosis factor-α, interleukin-6, and C-reactive protein. However, the precise role of aging in obesity and related metabolic disorders is unclear.

Animal models can help elucidate the relationship between aging, obesity, and metabolic disorders and can be used in pharmacological studies. One advantage of animal models is having precise control of factors. Furthermore, histological and biochemical studies, which are difficult to perform in humans, can be conducted. Rodents are the most common laboratory animals used in experimental models for the study of metabolic turnover of substances in the body[5,6]. To induce these metabolic disorders, various dietary, genetic, and pharmacological models have been developed at different ages. While many models display some of the signs of metabolic damage, few models can adequately and fully mimic the range of signs characterized in humans.

In this study, we sought to examine the potential effects of aging on high-fat diet (HFD)-induced obesity and its metabolic damage. We challenged mice of different ages with a defined period of HFD, a model that displays several correlations with human obesity.

2. Materials and methods

2.1. Animal models and diets

All experiments were approved by the Institutional Animal Care and Use Committee protocol of the MacKay Memorial Hospital. Six-week-old (young) male C57BL/6J mice were purchased from the National Laboratory Animal Center (Taipei, Taiwan) and allowed free access to standard chow (CHOW, 5010; LabDiet, PMI® Nutrition International, LLC, Brentwood, MO, USA) until 44 weeks of age (elderly)[7]. The light–dark cycle was 12:12, with a controlled environment (23°C, 50–60% relative humidity). Elderly (E) or young (Y) mice ($n = 6–10$/group) were randomly divided into either CHOW or HFD (45% Kcal, D12451; Research Diets, Inc., New Brunswick, NJ, USA) groups for 12 weeks (Table 1).

2.2. Blood sampling and analysis

Mice were fasted overnight before anesthetization. Blood was obtained by cardiac puncture and stored on ice for 30 minutes, and then separated by centrifugation (1000 rpm, 15 minutes) to obtain the serum. Levels of the following factors in serum were determined using a dry chemistry analyzer (FUJI DRI-CHEM NX500; Fujifilm, Japan): alanine aminotransferase (ALT), total cholesterol, and triglyceride. Enzyme-linked immunosorbent assays were performed for mouse insulin (EMD Millipore Co., USA) and mouse lipopolysaccharides (LPSs; CUSABIO Biotech Co., China).

2.3. Radioimmunoassay for insulin and homeostasis model assessment-estimated insulin resistance

For the fasting-based index, insulin sensitivity was calculated from the homeostasis model assessment-estimated insulin resistance (HOMA-IR) means of glucose and insulin values. HOMA-IR was calculated using the following formula[8]: HOMA-IR = [fasting insulin (mU/mL) × fasting glucose (mM/L)]/22.5

2.4. Body fat and lean mass analysis

Mice were kept anesthetized with 2% isoflurane in oxygen at 2 L/min on a scanning table. Fat and lean volumes in the anesthetized mice were analyzed by high-resolution *in vivo* microcomputed tomography (micro-CT; SkyScan 1076, SkyScan, Bruker, Kontich, Belgium) equipped with a tungsten target X-ray tube and a 0.5-mm Al filter. The system was operated at 50 kVp and 150 μA electric current. The equipment was adjusted to obtain the optimal trade-off between adequate contrast and resolution of the fat or lean deposits and a reasonable scan time. Total fat, visceral fat, and subcutaneous fat areas in mice were analyzed on the images from the level of the neck to the bottom using CTAn software (SkyScan, Bruker)[9].

2.5. Histopathological analysis of liver and adipose tissues

Liver and adipose tissues were fixed in 10% buffered neutral formalin and embedded in paraffin for subsequent hematoxylin–eosin staining. Hepatic pathology of NAFLD was evaluated based on the Kleiner Scoring System[10]. In brief, hematoxylin–eosin-stained liver sections were scored for steatosis (0–3), lobular inflammation (0–3), and hepatocyte ballooning (0–2). The sum of these findings was used to determine the NAFLD activity score (NAS).

2.6. Gut microbiota analysis

Stool was sampled at random from cages ($n = 4$/group) and immediately stored at −20°C for further analysis. The stool was used for DNA extraction by the E.Z.N.A. stool DNA kit (Omega Biotek, USA) according to the manufacturer's instructions. The concentration was determined using a NanoDrop 2000 Spectrophotometer (Thermo Scientific). DNA detection and amplification by real-time quantitative polymerase chain reaction (PCR) were

Table 1
Composition and energy content of the high-fat diet and standard chow.

Content (Kcal%)	Diets	
	HFD	CHOW
Fat %	45.0	12.8
Protein %	20.0	28.7
CHO %	35.0	58.5
Energy content (Kcal/kg)	4730	3080
Protein ingredients	Casein, L-cysteine	Fish meal, yeast, alfalfa meal
Fat ingredients	Soybean oil, lard	Pork fat, soybean oil,
CHO ingredients	Corn starch, maltodextrin, sucrose	Corn starch, wheat, soybean, oats

CHO = carbohydrate; CHOW = standard chow; HFD = high-fat diet.