

Increased Lipid Absorption and Transport in the Small Intestine of Zucker Obese Rats

Keizo Anzai^{1,*}, Koji Fukagawa², Ryuichi Iwakiri³, Kazuma Fujimoto³, Koichi Akashi⁴, and Patrick Tso⁵

¹Department of Endocrinology and Diabetes, Fukuoka University School of Medicine, Fukuoka, Jonan-Nanakuma 814-0180, Japan

²Department of Internal Medicine 1, Faculty of Medicine, Oita University, Oita, Yufu-Hazama 879-5593, Japan

³Department of Internal Medicine and Gastrointestinal Endoscopy, Saga Medical School, Saga, Saga-Nabeshima 849-8501, Japan

⁴Department of Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences, Fukuoka, Higashi-Maedashi 812-8582, Japan

⁵Department of Pathology and Laboratory Medicine, University of Cincinnati, Cincinnati, OH, USA

Received 8 January, 2009; Accepted 17 January, 2009

Summary The aim of this study was to compare the potency of intestinal lipid absorption in Zucker obese rats compared with Sprague-Dawley (SD) rats. Six male Zucker obese (fa/fa) and six male SD rats bearing intestinal lymph fistulae were used in this study. After 24 h recovery, rats were infused intraduodenally with a lipid emulsion containing 40 μmol triolein (labeled with glycerol tri[³H-oleate]), 7.8 μmol phosphatidyl choline in 3 ml phosphate buffered saline at a rate of 3 ml/h for 8 h. Lymph samples were collected and the radioactive lipid content determined. Apolipoprotein B (apo B) level in the lymph was evaluated. The Zucker obese rats transported more radioactive lipid into the lymph compared with the SD rats, particularly in the early phase of lipid absorption. Lymph apo B levels in the intestinal mucosa were significantly increased compared with the SD rats. In conclusion, this study indicated that lipid absorption and transport in Zucker obese rats is concomitant with increased apo B levels in the mesenteric lymph, indicating that the increase in lipid absorption may be responsible, at least in part, for obesity progression and hyperlipidemia.

Key Words: apolipoprotein, jejunum, hyperlipidemia, hyperphagia, obesity

Introduction

Zucker obese rats are hyperlipidemic with increased abdominal fat relative to lean littermates [1, 2]. However, the contribution of lipid absorption and transport in Zucker obese rats to their progressive obesity and hyperlipidemia is still unclear. We previously demonstrated that increased transport of absorbed lipid might be involved in hyper-

lipidemia in experimental diabetic rats [3]. Apolipoprotein (apo B) plays an important role in intestinal lipid transport to the lymph flow [4–6], and apo B is essential for the formation of triglyceride-rich lipoproteins and chylomicrons [7]. Lymph levels of apo B were not changed after lipid infusion in Sprague-Dawley (SD) rats [8]. The purpose of this study were to determine i) whether intestinal lipid absorption and lipid transport are increased in Zucker obese rats, and ii) whether the mesenteric lymph levels of apo B were increased compared with SD rats.

*To whom correspondence should be addressed.

Tel: 81-801-1011 Fax: 81-92-865-5656

E-mail: akeizo@fukuoka-u.ac.jp

Materials and Methods

Six male Zucker obese rats (fa/fa) (body weight; 450–550 g) and six male SD rats (body weight; 280–350 g), both aged 10–12 weeks, were used. All animal protocols were approved by the Institutional Animal Review Board of Saga Medical School. Each rat was fasted overnight before surgical procedures. Under halothane anesthesia, laparotomy was performed and the main mesenteric lymph duct was cannulated with a clear vinyl tubing (OD 0.8 mm) according to the method of Bollman, *et al.* [9], as described previously [8, 10]. A silicon infusion tube (OD 2.2 mm) was introduced about 2 cm along the duodenum through the fundus of the stomach. Postoperatively, the rat was infused via the intraduodenal tube with a glucose-saline solution (145 mM NaCl, 4 mM KCl, 0.28 M glucose) at a rate of 3 ml/h and allowed to recover in restraint cages for at least 24 h before lipid infusion.

Rats were infused with a lipid emulsion containing 0.354 g (400 μ l) of triolein, 1 μ Ci of glycerol tri[9,10(n)-³H]oleate, 68 mg of egg phosphatidylcholine (78 μ mol), and 570 μ mol of sodium taurocholate in 30 ml of phosphate-buffered saline (PBS). PBS contained 6.74 mM Na₂HPO₄, 16.5 mM NaH₂PO₄, 115 mM NaCl and 5 mM KCl, pH 6.4. The emulsion was infused at 3 ml/h for 8 h. Lymph was collected into precooled tubes for 1 h before the lipid infusion was started. This lymph sample was analyzed as the fasting lymphatic output. Subsequent lymph samples were collected at 2, 4, 6 and 8 h after starting the lipid infusion. Radioactivity was measured in an aqueous miscible scintillant (Opti-Fluor, Packard, Downers Grove, IL). The

samples were counted for 10 min in a liquid scintillation spectrometer (460 CD, Packard).

Apo B concentrations in the lymph samples were determined by electroimmunoassay (EIA), as described by Laurell [11], with modifications [8]. Gels for apo B EIA were made of 1% Indubiose agarose (Accurate Chemical & Scientific, Westbury, NY) and 3% polyethylene glycol in tris-tricine buffer containing 0.5%, by volume, of rabbit anti-rat apo B antiserum.

Data for lymph flow, radioactive ³H-triolein output and apo B lymph levels were evaluated by two-way analysis of variance, and multiple comparisons were carried out using the method of least significance.

Results

Lymph flow in the fasting state and after lipid infusion is shown in Table 1. Lymph flow increased 4 h after lipid infusion in both groups ($p < 0.05$). Basal lymph flow in the Zucker obese rat (2.5 ± 0.2 ml) was lower than in the SD rat (2.8 ± 0.2 ml), but not significantly. After lipid infusion, there was no difference in the lymph flow at each sampling time in both groups.

Changes in lymph radioactive lipid levels in the Zucker obese and SD rat are shown in Table 2. Lymph radioactive lipid levels are expressed as % of hourly infused radioactivity. After lipid infusion, the lymph radioactive lipid levels increased in the Zucker obese rat and the SD rat in a time dependent manner ($p < 0.01$ for both). The lymph radioactive lipid level in the Zucker obese rat reached a level at around the maximum at 2–4 h after the infusion, although

Table 1. Lymph flow before and after lipid infusion in Zucker obese rats and SD rats.

Fast	0–2 h	2–4 h	4–6 h	6–8 h
Zucker obese rats				
2.5 ± 0.2	2.1 ± 0.2	3.0 ± 0.3	3.8 ± 0.2^a	3.9 ± 0.3^a
SD rats				
2.8 ± 0.2	2.4 ± 0.3	3.3 ± 0.2	4.0 ± 0.3^a	4.0 ± 0.3^a

Values (ml/h) are expressed as means \pm SE. Fast: lymph flow at 1 h before the infusion. ^a $p < 0.05$, compared with Fast. Lymph flow increased at 4 h after the infusion in the both groups.

Table 2. Lymphatic radioactive triolein output after lipid infusion.

	0–2 h	2–4 h	4–6 h	6–8 h
Zucker obese rats				
$38.2 \pm 1.2\%$	$84.5 \pm 2.0\%^{a,b}$	$89.8 \pm 1.0\%^{a,b}$	$87.3 \pm 1.2\%^{a,b}$	
SD rats				
$30.2 \pm 1.4\%$	$64.1 \pm 2.0\%^a$	$80.4 \pm 1.5\%^a$	$79.4 \pm 1.9\%^a$	

Values (% of are hourly radioactive triolein infusion) expressed as means \pm SE. ^a $p < 0.01$, compared with lipid output at 0–2 h after infusion. ^b $p < 0.05$, compared with lipid output of SD rats at each time point.

Table 3. Mesenteric lymph apolipoprotein B levels before and after lipid infusion.

	Fast	0–2 h	2–4 h	4–6 h	6–8 h
Zucker obese rats					
	272 ± 14 ^a	340 ± 40 ^{a,b}	400 ± 25 ^{a,b}	550 ± 21 ^{a,b}	520 ± 28 ^{a,b}
SD rats					
	180 ± 21	120 ± 30	170 ± 15	190 ± 28	200 ± 22

Values ($\mu\text{g/h}$) are expressed as means \pm SE. Fast: lymph flow at 1 h before the infusion. ^a $p < 0.05$, compared with Fast. ^b $p < 0.01$, compared with apolipoprotein B levels of SD rats at each time point.

it reached the maximum at 6–8 h after the infusion in the SD rat. The lymph radioactive lipid level was significantly higher in the Zucker rats compared with the SD rats at all time points during the 8-h period ($p < 0.05$ for all).

Lymph apo B levels at each sampling time are shown in Table 3 for the Zucker obese rats and the SD rats. Lymph apo B levels in the fasting lymph of the Zucker rats were significantly higher compared with that of the SD rat ($p < 0.01$). The lymph apo B level in the Zucker rats increased immediately after the lipid infusion, and this increase continued over the 8-h sampling time ($p < 0.05$ in each). In contrast to the Zucker obese rat, the lymph apo B level of the SD rats did not increase after lipid infusion.

Discussion

Zucker obese rats have been used as animal model for genetical obesity. Several metabolic abnormalities of Zucker obese rats are common with human obese subjects. Zucker obese rats show hyperphagia [12], hyperlipidemia [13, 14], hyperinsulinemia [14, 15], and increased fat deposition [2]. The defective catabolism of chylomicrons in Zucker obese rats and overproduction of VLDL from the liver are considered to be the origin of hyperlipidemia in these rats [16–18]. However, the potency of lipid absorption and transportation to the mesenteric lymph of Zucker obese rats is largely unknown.

This study has provided some evidence for increased intestinal absorption and transport of lipids in Zucker obese rats compared with SD rats. The level of labeled triolein in the mesenteric lymph was significantly higher in Zucker obese rats compared with SD rats. Time-course analysis revealed that the lymph lipid level started to rise in the early phase after lipid infusion. These data indicate that Zucker obese rats absorb lipids more effectively than SD rats. These traits of Zucker obese rats may, in part, be related to the progression of obesity and hyperlipidemia. This hypothesis is supported by another study which found decreased fecal lipid and increased pancreatic lipase activity in Zucker obese rats [19]. There is the possibility that hyperphagia in Zucker obese rats affects pancreatic lipase activity and increases lipid absorption. To test this, additional experi-

ments involving pair feeding and food restriction are needed.

Apo B is necessary for chylomicron formation, and levels of Apo B are related to the number of chylomicrons [4, 7, 8]. The present study showed that the lymph apo B levels were higher in the fasting state in Zucker obese rats than in SD rats. There may be a large preformed pool of apo B in Zucker rats, contributing to the high apo B lymph level in the fasting stage. The Apo B level increased significantly after lipid infusion, and the higher lymph apo B level may be responsible for the higher lymph lipid level after lipid infusion. In contrast to the Zucker obese rats, the apo B level in the mesenteric lymph did not increase after lipid infusion in the SD rats, which is consistent with a previous study that showed no increment of apo B level in the mesenteric lymph or of apo B synthesis in the jejunal mucosa after lipid infusion [8].

In conclusion, Zucker obese rats absorbed and transported lipid more rapidly compared with the SD rats, and occurred concomitantly with increased mesenteric lymph levels of apo B. Increased lipid absorption and transport to the mesenteric lymph in Zucker obese rats may promote, at least in part, their obesity, hyperlipidemia and fat absorption.

References

- [1] Wang, C.S., Fukuda, N., and Ontko, L.A.: Studies on the mechanism of hypertriglyceridemia in the genetically obese Zucker rats. *J. Lipid. Res.*, **25**, 571–579, 1984.
- [2] Zucker, T.F. and Zucker, L.M.: Fat accretion and growth in the rat. *J. Nutr.*, **80**, 6–19, 1963.
- [3] Noda, T., Iwakiri, R., Fujimoto, K., Yosida, T., Utsumi, H., Sakata, H., Hisatomi, A., and Fujimoto, K.: Suppression of apoptosis is responsible for increased thickness of intestinal mucosa in streptozotocin-induced diabetic rats. *Metabolism*, **50**, 259–264, 2001.
- [4] Tso, P. and Fujimoto, K.: The absorption and transport of lipids by the small intestine. *Brain Res. Bull.*, **27**, 477–482, 1991.
- [5] van Greevenbroek, M.M. and de Bruin, T.W.: Chylomicron synthesis by intestinal cells *in vitro* and *in vivo*. *Atherosclerosis*, **141**, S9–S16, 1998.
- [6] Tanaka, A.: Postprandial hyperlipidemia and atherosclerosis. *J. Atheroscler. Thromb.*, **11**, 322–329, 2004.

- [7] Bhattacharya, S. and Redgrave, T.G.: The content of apolipoprotein B in chylomicron particles. *J. Lipid Res.*, **22**, 820–828, 1981.
- [8] Hayashi, H., Cardelli, J.A., Nutting, S., Bergstedt, S., and Tso, P.: Fat feeding increase size, but not number, of chylomicrons produced by small intestine. *Am. J. Physiol.*, **259**, G709–G719, 1990.
- [9] Bollman, J.L., Cain, J.C., and Grindlay, J.H.: Techniques for the collection of lymph from the liver, small intestine or thoracic duct of the rat. *J. Lab. Clin. Med.*, **33**, 1349–1352, 1948.
- [10] Ogata, S., Fujimoto, K., Iwakiri, R., Matsunaga, C., Ogawa, Y., Koyama, T., and Sakai, T.: Effect of polydextrose on absorption of triglyceride and cholesterol in mesenteric lymph fistula rats. *Proc. Soc. Exp. Biol. Med.*, **215**, 53–58, 1997.
- [11] Laurell, C.-B.: Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal. Biochem.*, **15**, 45–52, 1966.
- [12] Fukagawa, K., Sakata, T., Yoshimatsu, K., Fujimoto, K., and Shiraishi, T.: Disruption of light-dark of feeding and drinking behavior, and ambulatory activities induced by development of obesity in the Zucker rat. *Int. J. Obesity*, **12**, 481–490, 1987.
- [13] Azain, M.J., Fukudo, N., Yamamoto, M., and Ontko, A.: Contributions of fatty acid and sterol synthesis to triglyceride and cholesterol secretion by the perfused rat liver in genetic hyperlipemia and obesity. *J. Biol. Chem.*, **260**, 174–181, 1985.
- [14] Frisbee, J.C. and Delp, M.D.: Vascular function in the metabolic syndrome and the effects on skeletal muscle perfusion: lessons from the obese Zucker rat. *Essays Biochem.*, **42**, 145–161, 2006.
- [15] Thyfault, J.P.: Setting the stage: possible mechanisms by which acute contraction restores insulin sensitivity in muscle. *Am. J. Physiol.*, **294**, R1103–R1110, 2008.
- [16] Schonfeld, G. and Pflieger, B.: Overproduction of very low-density lipoproteins by the livers of genetically obese rats. *Am. J. Physiol.*, **220**, 1178–1181, 1971.
- [17] Kojima, K., Ogawa, A., Nakamura, R., and Kasai, M.: Effect of dietary medium-chain triglycerol on serum albumin and nitrogen balance in malnourished rats. *J. Clin. Biochem. Nutr.*, **2**, 45–49, 2008.
- [18] Redgrave, T.G.: Catabolism of chylomicron triacylglycerol and cholesteryl ester in genetically obese rats. *Biochem. Biophys. Res. Commun.*, **142**, 78–85, 1987.
- [19] Comai, K., Trscari, J., and Sullivan, A.C.: Differences between lean and obese Zucker rats: The effect of poorly absorbed dietary lipid on energy intake and body weight gain. *J. Nutr.*, **108**, 826–835, 1978.