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Dietary Protein Restriction Alters Lipid Metabolism and Insulin Sensitivity in Rats

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ABSTRACT : Dietary protein restriction affects lipid metabolism in rats. This study was performed to determine the effect of a low protein diet on hepatic lipid metabolism and insulin sensitivity in growing male rats. Growing rats were fed either a control 20% protein diet or an 8% low protein diet. Feeding a low protein diet for four weeks from 8 weeks of age induced a fatty liver. Expression of acetyl-CoA carboxylase, a key lipogenic enzyme, was increased in rats fed a low protein diet. Feeding a low protein diet decreased very low density lipoprotein (VLDL) secretion without statistical significance. Feeding a low protein diet down-regulated protein expression of microsomal triglyceride transfer protein, an important enzyme of VLDL secretion. Feeding a low protein diet increased serum adiponectin levels. We performed glucose tolerance test (GTT) and insulin tolerance test (ITT). Both GTT and ITT were increased in protein-restricted growing rats. Our results demonstrate that dietary protein restriction increases insulin sensitivity and that this could be due to low-protein diet-mediated metabolic adaptation. In addition, increased adiponectin levels may influences insulin sensitivity. In conclusion, dietary protein restriction induces a fatty liver. Both increased lipogenesis and decreased VLDL secretion has contributed to this metabolic changes. In addition, insulin resistance was not associated with fatty liver induced by protein restriction. (**Key Words :** Dietary Protein Restriction, Fatty Liver, Lipid Metabolism, Insulin Sensitivity)

INTRODUCTION

In young growing rats, dietary protein restriction alters lipid metabolism and causes a fatty liver (Singal et al., 1953; Morris et al., 1965). It has been reported that protein restriction increases food intake (White et al., 2000a) or shows no change in food intake (White et al., 2000b). In other studies, low protein diet decreases body weight and increases food intake and accumulates body fat (Begriche et al., 2006; Aparecida de França et al., 2009; Theys et al., 2009). Thus, the effects of protein restriction on food intake and lipid metabolism are still controversial. These inconsistencies may be related to many factors, such as the level and the time of the restriction, age, physiological state, and others. Thus, verification study for the effect of dietary protein restriction on growth, food intake, and metabolic changes is needed. Insulin resistance has been identified as a key factor in the development of and progression of a fatty liver (Marchesini and Forlani, 2002; Tilg and Hotamisligil, 2006). However, little is known about effect of dietary protein restriction on insulin sensitivity. This study was performed to understand the effect of low protein diet on hepatic lipid accumulation, metabolic changes, and insulin sensitivity in growing male rats.

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MATERIALS AND METHODS

Animals and diets

All experimental procedures involving animals were approved by the Chonnam National University (CNU) Institutional Animal Use and Care Committee. All procedures for animal management followed the standard operation protocols of CNU. Sprague-Dawley male rats (42 day-old, 170-190 g) were purchased from Orient Bio, Korea and were housed individually in carbonate cages and maintained in Animal house, Chonnam National University under a temperature-and humidity-controlled room on a 12-h light:12-h dark cycle for free access to food and water *ad libitum*. First 2 weeks, rats were given Standard lab chow for adaptation. Diets were purchased from Dytes, Inc, USA. Rats were divided into two groups (5 rats/group). They received either a control 20% protein diet or an 8% low protein diet, which was isocaloric and contained an adequate supply of choline and cysteine (Table 1). The

Table 1. Composition of experimental diets (g/kg)

Ingredient	Control diet (20P)	Low protein diet (8P)
Casein, high nitrogen	230	92
L-cystine	3	3
Sucrose	100	100
Cornstarch	354	492.901
Dyetrose	121.9	121.6
Soybean oil	70	70
t-butylhydroquinone	0.014	0.014
Cellulose	50	50
Mineral Mix #213025 (No Ca or P, reduced K)	35	35
Vitamin Mix #310025	10	10
Calcium carbonate (40.04% Ca)	12.495	12.495
Potassium citrate H_2O (K = 36.15%)	5.5	10.49
Potassium phosphate, mono (K = 28.73%, P = 22.76%)	5.6	0
Choline bitartrate	2.5	2.5
Total	1,000	1,000
Kcal/kg	3,750.22	3,755.08

formulation of the control diet was based on a modified AIN-93G diet (Dytes, USA). Rats were fed each diet during 28 days. During experimental period, food intake was monitored daily and body weight was recorded once in two days. At the end of the experimental period animals were sacrificed and blood and tissues were collected and weighed and tissue samples were quickly froze by liquid nitrogen and stored at -80°C.

Blood analyses

Whole serum parameters were analyzed by Green cross Reference Lab in Korea. Serum glucose was analyzed by Glucose II (HK) Reagents (Bayer, USA). HDL cholesterol was determined by enzymatic method using Direct HDL-Cholesterol kit (Bayer). LDL cholesterol was determined using LDL-cholesterol kit (Bayer). Total cholesterol was determined by enzymatic method using Cholesterol reagent (Bayer, USA). All cholesterol contents were analyzed by ADVIA 1650 (Bayer, Japan). Serum triglyceride (TG) was analyzed by enzymatic colorimetry using triglycerides reagent (Bayer). The result of TG was detected by ADVIA 1650 (BAYER, Japan). Serum free fatty acid was analyzed by enzymatic assay kit (Wako, USA). Serum adiponectin, insulin, leptin and glucagon levels were determined by Radio immuno assay (RIA) kit (LINCO'S Research, Inc. USA) using an antibody raised against rat.

Liver histology and triglyceride content

Liver specimens were fixed in 10% buffered formalin, equilibrated in 20% sucrose for 24 h at 4°C, and embedded in OCT (Optimal Cutting Temperature) compound. Frozen sections were stained with hematoxylin and eosin (H&E). To detect fat deposition in the liver, frozen sections were fixed for 10 min in neutral buffered 10% formalin, and then rinsed with distilled water, stained with 0.8% oil red O (Sigma-Aldrich, St. Louis, MO, USA) with 60% 2-propanol (Sigma-Aldrich) for 10 min, and then rinsed with distilled water.

Hepatic lipid was analyzed by extraction with chloroform/methanol (Folch et al., 1957). Liver triglyceride content was measured by enzymatic colorimetric methods after the total lipids were dissolved in isopropanol.

Determination of VLDL secretion

The rate of very-low-density lipoprotein (VLDL) secretion *in vivo* was determined as previously described (Chang et al., 1999). Briefly, in separate trial, experimental diets (5 rats/group) were fed for 28 days, and then we injected intravenously triton WR1339 (200 mg/kg BW), a lipoprotein lipase inhibitor. Blood was collected at 0, 1, 2, 3, and 4 h after injection. The concentration of triglyceride content in serum was determined by enzymatic spectrophotometric assay in Green Cross Reference Lab (Korea).

Glucose tolerance test (GTT) and insulin tolerance test (ITT)

In separate trial, experimental diets (8 rats/group) were fed for 28 days for GTT and 33 days for ITT, respectively, and then rats were subjected to GTT after an 8-h fast. Glucose (2 g/kg body weight) was administered intraperitoneally. Blood was drawn from a tail vein at 0, 10, 30, 60, 90, and 120 min after administration of the glucose, and glucose levels were measured.

Rats were subjected to ITT after a 6-h fast. Insulin (0.75 U/kg body weight) was injected intraperitoneally.

Blood was drawn from a tail vein at 0, 10, 30, 60, 120, and 180 min after the administration of insulin. Glucose was measured by Accu-CHEK® Active (Roche Diagnostics GmbH, Germany).

Western blotting

Tissues were homogenized in RIPA buffer containing a protease inhibitor. Samples were centrifuged and protein content in the supernatant was determined using the Bradford method. Protein samples were separated by SDS-PAGE and analyzed by immunoblotting, using antibodies purchased from Cell Signaling (USA). Blots were developed with secondary anti-rabbit or anti-mouse antibodies conjugated to horseradish peroxidase (Invitrogen) and the luminal chemiluminescence reagent (ECL; Amersham Biosciences, Sweden). The processed blots were then exposed to X-ray film.

Statistical analysis

Data from the two experimental groups were compared using Student's *t*-test.

RESULTS AND DISCUSSION

Growth parameters and fatty liver formation

Protein-restricted rats consumed more ($p = 0.03$) food, while there was no change in body weight by dietary protein restriction for 28 d (Table 2). Thus, food efficiency (food intake/weight gain) was decreased ($p = 0.05$; higher value) by dietary protein restriction.

Protein-restricted rats showed slightly higher total adipose tissue weights (sum of epididymal, subcutaneous, abdominal, and perirenal fat) with no statistical significance. In other study, low 10% dietary protein had no effect on body lipid composition (White et al., 2000a). This is in contrast to previous report that low dietary protein increases body fat (Thonney et al., 1987). An increase in body fat in low-protein–induced hyperphagic animals has also been previously observed (White et al., 1994). Low dietary protein has been suggested to increase energy expenditure (Rothwell et al., 1983). An increase in energy expenditure would use excess calories to generate heat rather than accumulate body fat. In our study, rats fed low 10% protein diet showed a lower efficiency of body weight gain, suggesting that extra energy may be used to generate heat. The reason that some animals consumed more low protein diet gain fat, whereas others appear to increase energy expenditure, is not known.

We found that protein restriction (PR) induced hepatic steatosis (fatty liver) by examining liver section with H&E and oil red O staining (Figure 1A). Correspondingly, PR increased ($p<0.01$) hepatic lipid and triglyceride contents (Figure 1B). Previously, feeding either a low-casein diet supplemented with sulfur-containing amino acids or a diet containing suboptimal levels of threonine causes fatty liver (Singal et al., 1953; Morris et al., 1965). Our study confirms that protein restriction induces a fatty liver.

Serum metabolites and hormones

We measured serum metabolic parameters. Serum triglyceride, glucose, free fatty acid, total cholesterol and HDL cholesterol levels were not significantly changed by protein restriction (Table 3). Interestingly, protein restriction decreased LDL cholesterol levels. Epidemiological and prospective studies have generally shown a direct relationship between total cholesterol and LDL-cholesterol and cardiovascular disease (Therond, 2009; Ascaso, 2010). Thus, dietary protein restriction may show beneficial effect on blood cholesterol parameters.

We measured serum metabolic hormones. Serum

Table 2. Effect of dietary protein restriction on growth and body parameters

Parameter	20P	8P	p-value
Growth parameters			
Body weight at d1 (g)	259.2±8.7	258.4±6.6	0.87
Body weight at d28 (g)	468.2±10.6	468.8±18.8	0.97
Total food intake[1] (g)	687.5±45.8	773.8±64.9	0.03
Food intake/weight gain	3.30±0.19	3.71±0.27	0.05
Body parameters			
Epididymal fat (g)	8.08±2.94	8.68±2.96	0.79
Subcutaneous fat (g)	10.46±3.03	14.16±5.61	0.30
Visceral fat (g)	9.98±2.15	11.72±2.82	0.35
Perirenal fat (g)	1.58±0.40	1.68±0.46	0.79
Total fat (g)	30.1±7.93	36.24±10.91	0.40
Liver (g)	17.66±2.43	19.42±2.36	0.09

[1] Total food intake = Sum of food intake from d1 through d27. Values are expressed as means±standard deviation (n = 5).

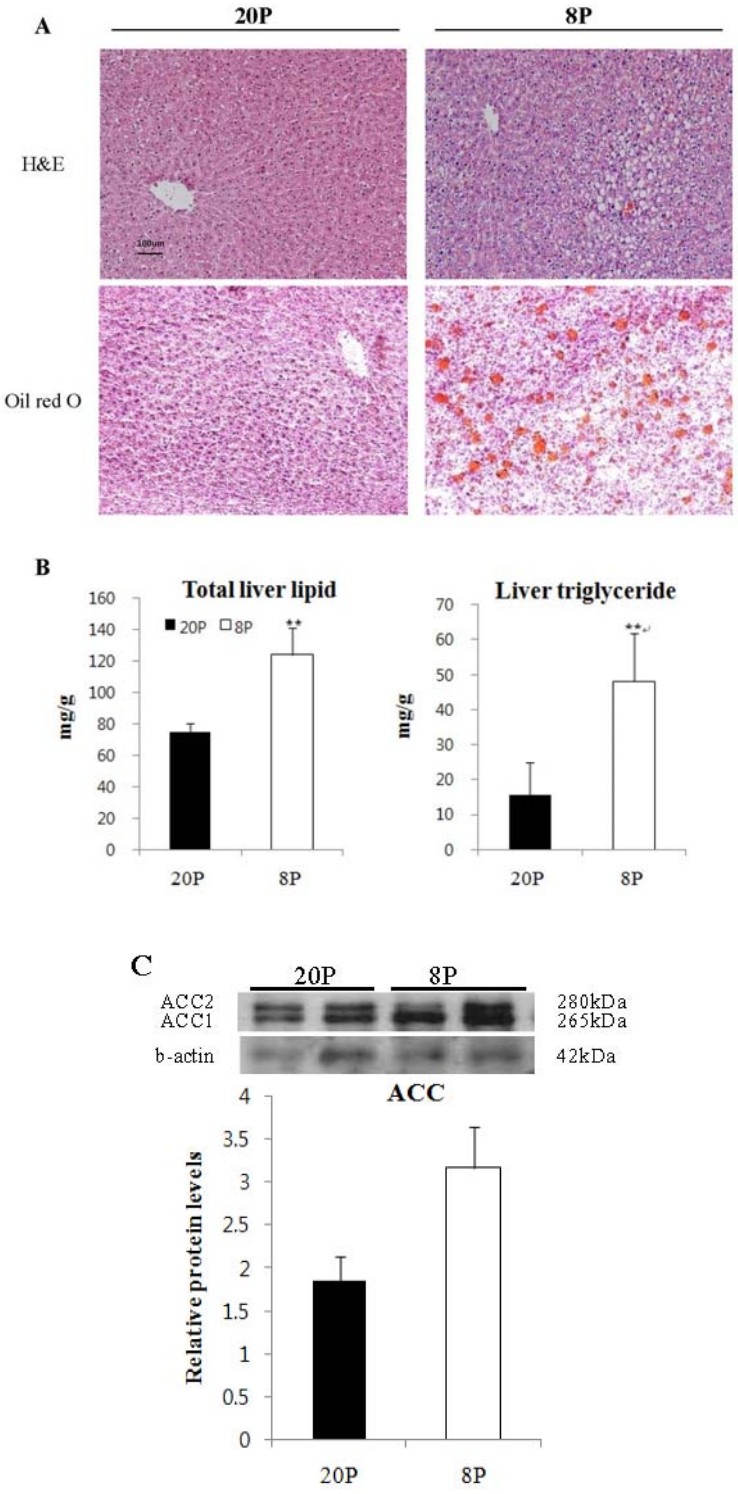

Figure 1. A fatty liver development and hepatic lipid and triglyceride contents in male rats fed with either a 20% control-protein diet (20P) or an 8% low-protein diet (8P). (A) Liver sections were stained with hematoxylin and eosin (H&E) and oil red O. The magnification for H&E and oil red O was ×200. The scale bars represent 100 μm (×200). (B) The hepatic lipid and triglyceride contents. (C) Protein levels of acetyl CoA carboxylase (ACC) were determined by western blotting and normalized with β-actin. Values are the mean+SD (n=3). Only three out of 5 rats were used for ACC Western. ** $p<0.01$.

Table 3. Effect of dietary protein restriction on serum metabolites and hormones

Parameters	20P	8P	p-value
Metabolites			
Total cholesterol (mg/dl)	95.4±7.8	77.0±34.4	0.31
HDL cholesterol (mg/dl)	24.6±1.1	23.0±11.3	0.78
LDL cholesterol (mg/dl)	9.2±1.3	6.4±2.6	0.05
Triglyceride (mg/dl)	161.6±55.4	212.6±122.2	0.48
Glucose (mg/dl)	183.2±36.0	220.6±31.5	0.16
Free fatty acid (μEq/L)	624.8±126.7	763.0±327.2	0.27
Hormones			
Adiponectin (μg/ml)	6.50±1.01	11.10±3.20	0.02
Glucagon (pg/ml)	56.4±26.2	39.8±11.0	0.13
Insulin (ng/ml)	1.74±0.65	1.41±.10	0.58
Leptin (ng/ml)	10.46±3.53	13.12±5.54	0.45

Values are expressed as means±standard deviation (n = 5).

glucagon, insulin, and leptin levels were not changed by protein restriction (Table 3). Other study also reported no change in leptin levels in lambs fed low protein diet (Yan et al., 2010). Interestingly, protein restriction increased ($p = 0.02$) serum adiponectin levels. Previously, serum adiponectin levels were elevated twofold when C57BI/6 mice were given methionine-choline deficient (MCD) diet for 4 weeks (Ikejima et al., 2007). Adiponectin regulates whole-body lipid partitioning. It exerts insulin-sensitizing effects in the liver, skeletal muscle, and adipose tissue (Marra and Bertolani, 2009). Adiponectin improves insulin signaling via inhibition of protein tyrosine phosphatase PTP1B (Fiaschi et al., 2007; Kim et al., 2007). We found that protein restriction increased insulin sensitivity (Figure 3A and 3B). Therefore, increased circulating adiponectin of rats fed low protein diet may has a role to increase insulin sensitivity. Serum adiponectin was also increased in db/db mice that received a MCD diet plus diacylglycerol acyltransferase 2-antisense oligonucleotide treatment to block triglyceride biosynthesis, resulting in decreasing fatty acid disposal (Yamaguchi et al., 2007). They have also suggested that adiponectin might be involved in increasing insulin sensitivity under severe hepatic lipotoxicity by failure of fatty acid disposal.

Protein restriction alters lipogenesis and VLDL secretion

Several factors such as an increased de novo lipogenesis and an impaired secretion of VLDL in the liver are responsible for the development of non-alcoholic fatty liver disease (Postic and Girard, 2008). First, we examined whether protein restriction affected expression of acetyl CoA carboxylase (ACC), a major lipogenic enzyme. Western blotting showed that protein-restricted rats have higher ACC protein level (Figure 1C). Previous study showed positive correlation between ACC protein levels and its enzymatic activity in rat liver (Atkinson et al., 2002). This result indicates that lipogenesis contributes to fatty liver induced by protein restriction.

Studies have suggested that a failure in VLDL secretion is one of contributing factors for hepatic fat accumulation (Minehira et al., 2008; Cano et al., 2009). We examined hepatic gene expression for VLDL secretion. Protein restriction showed down-regulation ($p<0.05$) of hepatic protein levels of microsomal triglyceride transfer protein (MTP) (Figure 2A). We examined *in vivo* VLDL secretion in protein-restricted growing male rats. Interestingly, we found that low-protein diet decreased VLDL secretion at all time points with no statistical significance (Figure 2B). Our data demonstrate that impairment of VLDL secretion is involved in hepatic steatosis. Recently, dysfunctional VLDL synthesis and release was suggested to be a key factor in NASH pathogenesis in human subjects (Fujita et al., 2009).

Protein restriction increases insulin sensitivity

Several studies demonstrated that insulin sensitivity decreased in obese and diabetic people. Insulin resistance has been identified as a key factor in the development of and progression of a fatty liver (Marchesini and Forlani, 2002). In present study, we found that low-protein diet increased hepatic lipid contents and induced fatty liver.

In a separate experiment, we first measured insulin sensitivity by GTT (Figure 3A) at 28 days after feeding. Five days later, we measured ITT (Figure 3B) at 33 days after feeding within same rats. We assumed that this minor difference of feeding duration has not affect effect of protein restriction on insulin sensitivity because we have studied chronic long-term effect of nutrition, but not acute short-term effect. Duration of feeding trial in rats with low protein diet varies between 12 days (White et al., 2000a), 28 days (Singal et al., 1953), and 14 and 28 days (Morris et al., 1965). Our results show that dietary protein restriction

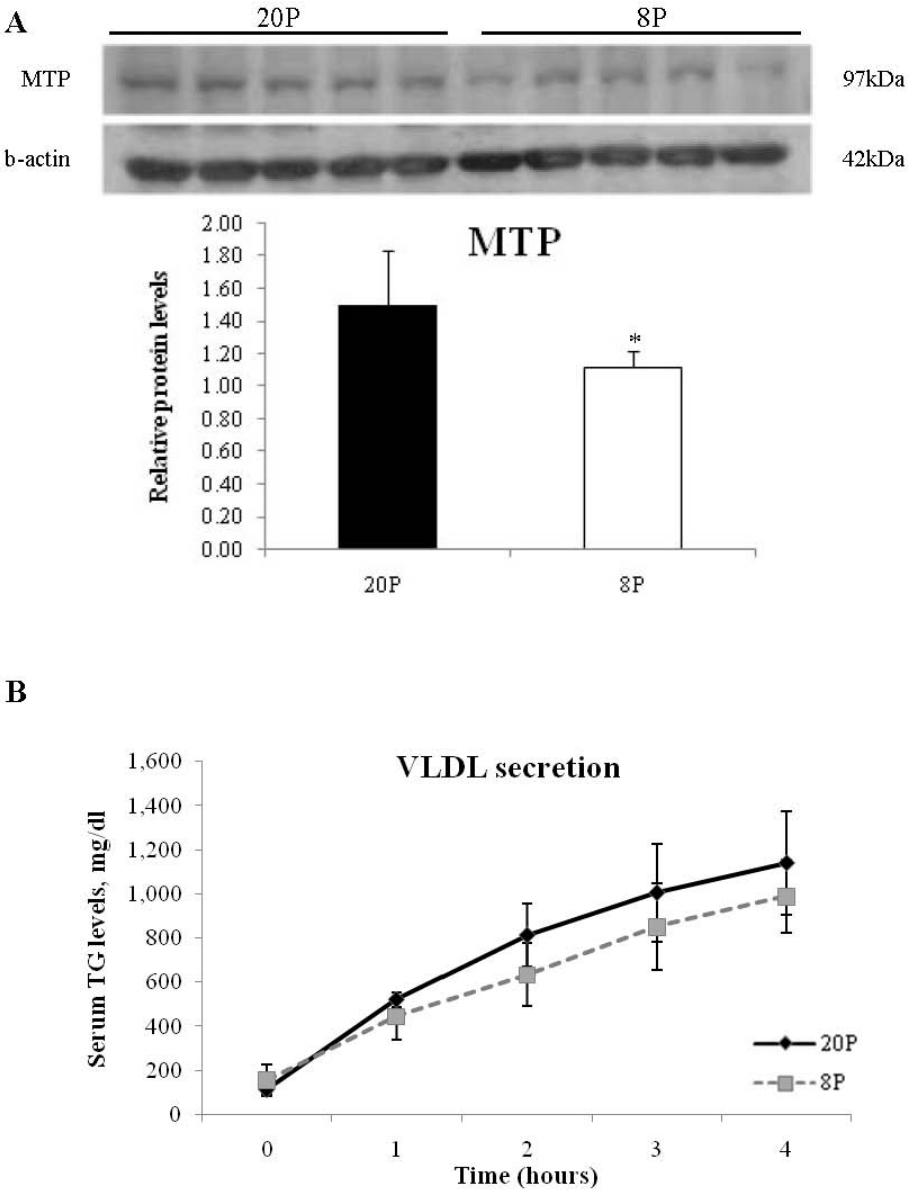

Figure 2. *In vivo* VLDL secretion and hepatic MTP protein expression in male rats fed with either a 20% control-protein diet (20P) or and 8% low-protein diet (8P). (A) Protein levels of microsomal triglyceride transfer protein (MTP) were determined by Western blotting and normalized with β-actin. (B) At 28 days after feeding, rats were fasted for 6th, and blood samples were taken at time zero and every hour for 4 h after Triton WR 1339 (a lipoprotein lipase inhibitor) intravenous injection, and serum triglyceride levels was measured. Values are expressed as means+standard deviation (n = 5). * $p<0.05$.

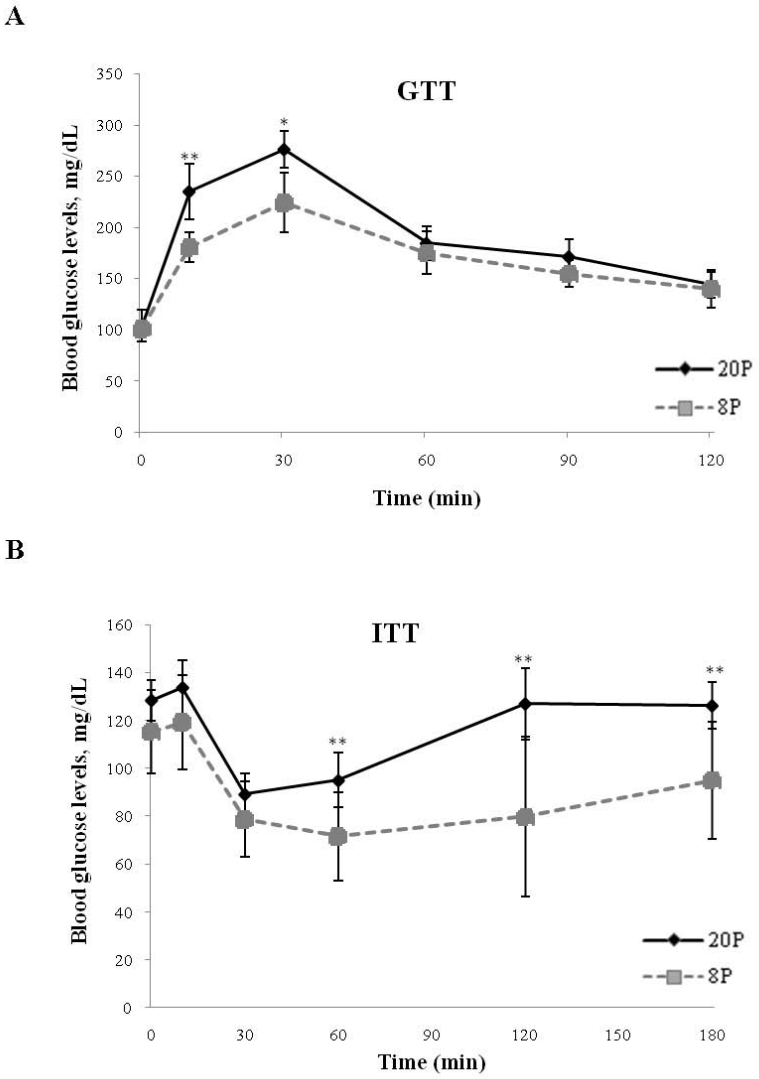

Figure 3. Insulin sensitivity of male rats fed with either a 20% control-protein diet (20P) or an 8% low-protein diet (8P). (A) For glucose tolerance test (GTT) at 28 days after feeding, rats were fasted for 8 h and infected 2 g glucose/kg of body weight intraperitoneally. (B) For insulin tolerance test (ITT) at 33 days after feeding, rats were fasted for 4 h and injected 0.75 U insulin/kg of body weight. After injection, blood glucose levels were analyzed at each time point. Values are expressed as mean±standard deviation (n = 8). * $p<0.05$, ** $p<0.01$.

increases insulin sensitivity and this could be due to low-protein diet-mediated metabolic adaptation. Increased adiponectin levels may positively influences insulin sensitivity. This indicates that insulin resistance was not associated with fatty liver in our model. An additional study is warranted to understand relationship between insulin sensitivity and adiponectin signaling in rats fed low-protein diet.

In conclusion, our study shows that dietary protein restriction induces a fatty liver. Our data also demonstrate that both increased lipogenesis and decreased VLDL secretion has contributed to hepatic lipid accumulation. In addition, insulin resistance was not associated with development of hepatic steatosis induced by protein restriction.

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REFERENCES

Aparecida de Franca, S., M. P. Dos Santos, M. A. Garofalo, L. C. Navegantes, C. Kettelhut Ido, C. F. Lopes and N. H. Kawashita. 2009. Low protein diet changes the energetic balance and sympathetic activity in brown adipose tissue of growing rats. Nutrition 25:1186-1192.

Ascaso, J. F. 2010. Advances in cholesterol-lowering interventions. Endocrinol. Nutr. 57:210-219.

Atkinson, L. L., S. E. Kelly, J. C. Russell, J. Bar-Tana and G. D. Lopaschuk. 2002. MEDICA 16 inhibits hepatic acetyl-CoA carboxylase and reduces plasma triacylglycerol levels in insulin-resistant JCR: LA-cp rats. Diabetes 51:1548-1555.

Begriche, K., A. Igoudjil, D. Pessayre and B. Fromenty. 2006. Mitochondrial dysfunction in NASH: causes, consequences and possible means to prevent it. Mitochondrion 6:1-28.

Cano, A., F. Ciaffoni, G. M. Safwat, P. Aspichueta, B. Ochoa, E. Bravo and K. M. Botham. 2009. Hepatic VLDL assembly is disturbed in a rat model of nonalcoholic fatty liver disease: is there a role for dietary coenzyme Q? J. Appl. Physiol. 107:707-717.

Chang, B. H., W. Liao, L. Li, M. Nakamuta, D. Mack and L. Chan. 1999. Liver-specific inactivation of the abetalipoproteinemia gene completely abrogates very low density lipoprotein/low density lipoprotein production in a viable conditional knockout mouse. J. Biol. Chem. 274:6051-6055.

del Rincon, J. P., K. Iida, B. D. Gaylinn, C. E. McCurdy, J. W. Leitner, L. A. Barbour, J. J. Kopchick, J. E. Friedman, B. Draznin and M. O. Thorner. 2007. Growth hormone regulation of p85alpha expression and phosphoinositide 3-kinase activity in adipose tissue: mechanism for growth hormone-mediated insulin resistance. Diabetes 56:1638-1646.

Fiaschi, T., F. Buricchi, G. Cozzi, S. Matthias, M. Parri, G. Raugei, G. Ramponi and P. Chiarugi. 2007. Redox-dependent and ligand-independent trans-activation of insulin receptor by globular adiponectin. Hepatology 46:130-139.

Folch, J., M. Lees and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Fujita, K., Y. Nozaki, K. Wada, M. Yoneda, Y. Fujimoto, M. Fujitake, H. Endo, H. Takahashi, M. Inamori, N. Kobayashi, H. Kirikoshi, K. Kubota, S. Saito and A. Nakajima. 2009. Dysfunctional very-low-density lipoprotein synthesis and release is a key factor in nonalcoholic steatohepatitis pathogenesis. Hepatology 50:772-780.

Ikejima, K., K. Okumura, K. Kon, Y. Takei and N. Sato. 2007. Role of adipocytokines in hepatic fibrogenesis. J. Gastroenterol. Hepatol. Suppl. 1:S87-92.

Kim, J. Y., E. van de Wall, M. Laplante, A. Azzara, M. E. Trujillo, S. M. Hofmann, T. Schraw, J. L. Durand, H. Li, G. Li, L. A. Jeilcks, M. F. Mehier, D. Y. Hui, Y. Deshaies, G. I. Shulman, G. J. Schwartz and P. E. Scherer. 2007. Obesity-associated improvements in metabolic profile through expansion of adipose tissue. J. Clin. Invest. 117:2621-2637.

Marchesini, G. and G. Forlani. 2002. NASH: from liver diseases to metabolic disorders and back to clinical hepatology. Hepatology 35:497-499.

Marra, F. and C. Bertolani. 2009. Adipokines in liver diseases. Hepatology 50:957-969.

Minehira, K., S. G. Young, C. J. Villanueva, L. Yetukuri, M. Oresic, M. K. Hellerstein, R. V. jr Farese, J. D. Horton, F. Preitner, B. Thorens and L. Tappy. 2008. Blocking VLDL secretion causes hepatic steatosis but does not affect peripheral lipid stores or insulin sensitivity in mice. J. Lipid Res. 49:2038-2044.

Morris, L., D. Arata and D. C. Cederquist. 1965. Fatty livers in weanling rats fed a low protein, threonine-deficient diet. I. Effect of various diet fats. J. Nutr. 85:362-366.

Postic, C. and J. Girard. 2008. The role of the lipogenic pathway in the development of hepatic steatosis. Diabetes Metab. 34:643-648.

Rothwell, N. J., M. J. Stock and R. S. Tyzbir. 1983. Mechanisms of thermogenesis induced by low protein diets. Metabolism 32:257-261.

Singal, S. A., S. J. Hazan, V. P. Sydenstricker and J. M. Littlejohn. 1953. The production of fatty livers in rats on threonine-and lysine-deficient diets. J. Biol. Chem. 200:867-874.

Therond, P. 2009. Catabolism of lipoproteins and metabolic syndrome. Curr. Opin. Clin. Nutr. Metab. Care 12:366-371.

Theys, N., T. Bouckenooghe, M. T. Ahn, C. Remacle and B. Reusens. 2009. Maternal low-protein diet alters pancreatic islet mitochondrial function in a sex-specific manner in the adult rat. Am. J. Physiol. Regul. Integr. Comp. Physiol. 297:1516-1525.

Thonney, M. L. amd D. A. Ross. 1987. Composition of gain of rats fed low or high protein diets and grown at controlled rates from 80 to 205 grams. J. Nutr. 117:2135-2141.

Tilg, H. and G. S. Hotamisligil. 2006. Nonalcoholic fatty liver disease: Cytokine-adipokine interplay and regulation of insulin resistance. Gastroenterology 131:934-945.

White, B. D., B. He, R. G. Dean and R. J. Martin. 1994. Low protein diets increase neuropeptide Y gene expression in the basomedial hypothalamus of rats. J. Nutr. 124:1152-1160.

White, B. D., M. H. Porter and R. J. Martin. 2000a. Effects of age on the feeding response to moderately low dietary protein in rats. Physiol. Behav. 68:673-681.

White, B. D., M. H. Porter and R. J. Martin. 2000b. Protein selection, food intake, and body composition in response to the amount of dietary protein. Physiol. Behav. 69:383-598.

Yamaguchi, K., L. Yang, S. McCall, J. Huang, X. X. Yu, S. K. Pandey, S. Bhanot, B. P. Monia, Y. X. Li and A. M. Diehl. 2007. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. Hepatology 45:1366-1374.

Yan, X., F. Zhang, D. Li, X. Zhu and Z. Jia. 2010. Effects of chromium on energy metabolism in lambs fed with different dietary protein levels. Asian-Aust. J. Anim. Sci. 23:205-212.