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The Innate Differences in the Adipocyte Membrane Fatty Acid Composition Could Be Modified By Dietary Fats in Obese Mice

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Abstract: *Obesity-related insulin resistance is the risk factor for developing type 2 diabetes mellitus. The fatty acid composition of plasma membrane is known as a mediator to affect insulin action. This study aimed to investigate the differences in the adipocyte membrane fatty acid profile between genetically obese (ob/ob) mice and their lean (+/?) counterparts. In addition, the influence of dietary manipulation with different polyunsaturated/saturated (P/S) fatty acid ratio was also studied. The results showed that ob/ob mice had lower unsaturated (18:1, 18:2) and higher saturated (16:0, 18:0) fatty acids in their adipocyte membranes compared to those of their lean counterparts. Moreover, the unsaturation index of the adipocyte membrane in ob/ob mice that fed a high-fat diet (P/S=0.6) was significantly decreased. In summary, our data indicated that the adipocyte membrane fatty acid composition of ob/ob mice was innately different from their lean counterparts and could be modified by dietary P/S ratio.*

Keywords: *Adipose, Insulin resistance, Obesity, P/S ratio, Unsaturation index*

I. Introduction

Obesity, a worldwide public health problem, increases the risk for developing type 2 diabetes mellitus and cardiovascular diseases [1]. Insulin resistance (IR), which is common in obese subjects, is attributed to defects in the pathway of insulin action in target tissues, like skeletal muscle, liver and adipose tissue (AT). Although AT accounts for only 10% of glucose uptake in general, it will consume more glucose up to 50% during obesity-developing period [2]. AT also may release excess fatty acids (FA) at disease state leading to ectopic lipid storage, as in fatty liver [3]. In addition, AT plays an active endocrine role sensing metabolic signals and regulating lipid mobilization in other organs by secreting certain adipokines. For example, adiponectin can reduce hepatic lipid content by stimulating FA oxidation. Therefore, IR may originate in AT and is followed by an increased efflux of FA, with secondary effects causing or exaggerating IR in liver and muscle tissues [4].

The physicochemical property of plasma membrane is largely determined by the nature of the FA within the phospholipid bilayer, which in turn affects the activities of various membrane-bound enzymes and hormonal responsiveness [5]. Membrane FA composition and its fluidity may influence several processes related to IR, including hormone binding, signal transduction, and availability of precursors for lipid metabolism [6]. In general, high dietary saturated FA (SFA) induces IR, whereas n-3 polyunsaturated FA (PUFA) prevents IR. Actually, IR is known closely related to the reduction in dietary long chain PUFA, particularly arachidonic acid [7]. Although membrane FA profile usually reflects the FA composition of the diet [8], membrane structural lipids appear to be more resistant to dietary modification [9].

As described above, high dietary fat intake or IR can alter the membrane FA profile, and this alteration is extensive enough to change membrane fluidity and affect insulin activity. However, the role that membrane FA profile of the adipocyte (AD) may play on obesity remains to be explored. The genetically obese (ob/ob) mouse, caused by inherent leptin deficiency and characterized by obesity, reduced thermogenesis, and hyperinsulinemia, is a widely used experimental model for studies on obesity and type 2 diabetes mellitus [10]. Since the AD membrane FA composition of the ob/ob mouse has yet been fully illustrated, the purpose of this study was to compare the difference in AD membrane FA profile between ob/ob mice and their lean counterparts. In addition, the dietary influence of altered PUFA/SFA ratio (P/S ratio) on AD membrane FA composition was also examined.

II. Materials And Methods

1. Animals: Genetically obese (ob/ob) mice and their lean controls (+/?) were obtained from C57BL/6J-ob/+ breeding pairs purchased from the Jackson Laboratory (Bar Harbor, ME, USA). The breeding pairs were housed in solid-bottomed cages, with wood shavings for bedding. Pregnant dams were removed from the breeding cage and placed in a separate cage. Pups were weaned at about 21 days of age, and only litters containing 5 to 7 pups were used in this study. Throughout the study, mice were kept at a temperature

(22±1℃)- and humidity (50±5%)-controlled room with a 12-h light/12-h dark cycle. Mice had free access to diet and distilled water. Food intake and body weight were recorded twice a week. All chemicals, unless specifically indicated, were purchased from Sigma (St. Louis, MO, USA). A protocol for animal care procedure according to the National Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology, Executive Yuan, Taiwan) was approved by the Overseas Chinese University Research Management and Review Committee (OCU-ea102052).

2. Experiment 1: Mice of both phenotypes (obese and lean) that were fed a standard laboratory chow (Purina #5001, St. Louis, MO, USA, containing 10.7% fat, which including 1.6% SFA, 1.6% MUFA, 1.4% PUFA, P/S ratio=0.9) beginning from their weanling time till their age of 12 weeks old were used. Each group contained 8 mice. By using a online software "Raosoft Sample Size Calculator", a sample size of 8 was selected to ensure a desired analysis power of 0.9 for 2-sided tests at α=0.05.
3. Experiment 2: Sixteen obese mice at 8 weeks of age were divided into 2 groups and assigned to receive either a high-fat diet (P/S=0.6) or a low-fat diet (P/S=1.2) for another 4 weeks. Slightly modified diets (Table 1) were prepared based on the AIN-93 standard [11].
4. Body fat and blood biochemical measurements: Mice at 12 weeks of age (experiment 1) or after the completion of a 4-week dietary treatment (experiment 2) were killed under anesthesia after an overnight fasting (in order to reduce the effects of diet intake on blood constituents). Body fat content was determined by a body composition analyzer (EM-SCAN Inc., Springfield, IL, USA). Their trunk blood samples were collected for the measurements of triacylglycerol (TAG), total cholesterol (CHOL) and high-density lipoprotein cholesterol (HDL-C), and were determined by the enzymatic method by using commercial kits obtained from Boehringer Mannheim (Mannheim, Germany). After measuring body fat content and collecting blood sample, gonadal AT in both sides of each mouse were removed.

Table 1. Composition of two dietary P/S ratios supplied for the obese mice

	Low-fat (P/S=1.2)	High-Fat (P/S=0.6)
Gross energy (kcal/g)	4.16	6.30
Percentage of energy:		
Protein	20%	20%
Fat	10%	60%
Carbohydrate	70%	20%
Diet compositing:		
Casein	19.7%	18.4%
Soy bean oil	5.0%	5.9%
Corn starch	66.4%	14.7%
Methyl cellulose	4.0%	6.3%
Mineral mixture	4.0%	6.3%
Vitamin mixture	0.4%	0.6%
Methionine	0.3%	0.5%
Choline	0.2%	0.3%
Tallow	0.0%	47.0%
The mineral and vitamin mixtures were purchased from ICN Biomedicals (Costa Mesa, CA, USA). Every nutrient was determined by percentage of weight. P/S ratio=Ratio between polyunsaturated and saturated fatty acids.		

5. Membrane isolation and FA determination: AD were prepared by the method described previously with slight modification [12]. In brief, 10 mL HEPES-sucrose buffer (20 mM HEPES, 255 mM sucrose, 1 mM EDTA, pH=7.4) containing protease inhibitors was added into AT, and homogenized under iced water. Then, the homogenate was centrifuged and the supernatant was separated from the pellet and fat cake, and centrifuged at 150000g for 2 h at 4℃. The pellet (total membrane fraction) was resuspended in HEPES-sucrose buffer, and recentrifuged for 1 h at 95000g. The membrane fraction that settled at the interface was collected, diluted and washed. The resulting pellet was suspended in Tris-EDTA buffer. Membrane lipid was extracted by following the method previously described [13] with methanol and chloroform containing 50 mg/L 2,6-dibutyl-P-cresol to prevent lipid peroxidation. The organic phase was evaporated to dryness, and the lipid residue was dissolved in acetonitrile. FA were isolated and conducted directly to the reverse-phase high performance liquid chromatography analysis (RP-HPLC). A μBondapak column (Waters fatty acid analysis column, 4x300 mm, Waters, Milford, MA, USA) was selected and the mobile phase composed of CH3CN: THF: H2O=45: 20: 35. HPLC analysis was performed by following the method previously described and according to manufacturer's instruction [14].
6. Statistical analyses: All data were presented as the mean±SD. The data of FA were calculated as a percentage weight basis. Statistical analyses of the results were conducted by analysis of variance and unpaired Student's *t*-test with a commercial package, KaleidaGraph 3.6 (Synergy Software, Reading, PA,

USA). The difference was considered to be significant when *P* value was less than 0.05.

III. Results

As expected, ob/ob mice had significantly higher food intake, body weight and body fat content compared to their lean counterparts. Plasma levels of TAG and total CHOL were increased, while HDL-C was decreased in ob/ob mice (Table 2). The AD membrane FA composition was markedly different between ob/ob mice and their lean counterparts. The percentages of SFA (C16:0, C18:0) were higher, but C18:1 and C18:2 were lower in ob/ob mice than those of their lean controls. However, arachidonic acid (C20:4) did not differ between both phenotypes. Unsaturation index (UI), which calculated by summing the percentage of each FA in a sample multiplied by the number of double bonds in that FA, was significantly reduced in ob/ob mice.

Table 2. Body weight, body fat content, plasma lipids levels, and adipocyte membrane fatty acid composition in obese mice and their lean counterparts at 12 weeks of age

	Lean (n=8)	Obese (n=8)
Body weight (g)	27.0±1.6	52.3±2.9*
Body fat content (%)	25.0±3.3	40.6±3.7*
Plasma lipid (mg/dl)		
TAG	90.5±15.2	166.4±36.3*
Total-CHOL	108.5±46.3	198.5±38.6*
HDL	29.3±10.0	19.6±3.1*
Membrane FA (%)		
16:0 (Palmitic acid)	20.1±0.8	22.6±0.6*
18:0 (Stearic acid)	23.2±0.9	27.2±1.1*
18:1 (Oleic acid)	16.2±0.6	13.9±0.9*
18:2 (Linoleic acid)	34.9±1.5	30.6±1.4*
20:4 (Arachidonic acid)	5.6±0.5	5.7±0.8
UI	108.4±6.8	97.9±8.2*

Mean ± SD. * indicated that there was a significant difference ($P<0.05$) between groups.
UI: unsaturation index is calculated by summing the percentage of each fatty acid in a sample multiplied by the number of double bonds in that fatty acid.

Table 3 shows that ob/ob mice fed with a high-fat diet (P/S=0.6) for 4 weeks had higher body weight and body fat content than that of ob/ob mice fed with a low-fat diet (P/S=1.2). In addition, plasma TAG was increased, but HDL-C was decreased in these high-fat fed mice. AD membrane FA profile was markedly affected by dietary P/S ratio. High-fat fed ob/ob mice had higher proportion of SFA (C16:0, C18:0), but lower proportion of C18:2 in their AD membrane than that of low-fat fed ob/ob mice. UI was also significantly reduced in ob/ob mice fed with a high-fat diet.

IV. Discussion

Insulin resistance (IR) hampers the normal role of insulin, which causes increased muscle glucose uptake and cessation of hepatic glucose production. In AD with IR, the process of TAG synthesis is decreased, while lipolysis remains unchecked, resulting in even higher levels of blood FA [3,4]. Furthermore, experimental diabetic rodents are found to have altered membrane phospholipid composition in their AD or skeletal muscle cells [5-8,15,16]. In our study, ob/ob mice had higher SFA, but lower UFA in their AD membrane than their lean counterparts, and the resulting reduced membrane fluidity should exacerbate the action of insulin. Once ingested and absorbed, FA are either incorporated into membrane lipids, into storage as TAG, or directly oxidized [5]. Membrane n-3 PUFA has been shown to be strongly influence by dietary composition [8]. For example, linoleic acid (C18:2) can not be synthesized by the body and must be provided from the diet, and serves as a precursor for arachidonic acid (20:4). Thus, the higher SFA in AD membrane of ob/ob mice was due to the increased activity of acetyl coenzyme A, which provided more SFA incorporated into membrane. Lower C18:2 but similar C20:4 in AD membrane of ob/ob mice suggested that this alteration was not resulted from the defects in the activities of desaturase and elongase enzyme systems. Based on the facts that Purina #5001 rodent chow contains less amounts of C18:2 (12.2 g/kg diet) and ob/ob mice have higher diet intake (hyperphagia) than their lean counterparts, the possibility that the decrease in C18:2 was caused by reduced food consumption could be excluded. Whether decreased levels of C18:1 and C18:2 in AD membrane of ob/ob mice are due to that these FA were more easily to be oxidized and removed from the membrane or by more increased corporation into intracellular TAG remains unknown and needs further study. Nevertheless, our data elucidated that AD membrane FA composition of ob/ob mice was innately different from their lean counterparts at their 12 weeks of age, the time when the obesity phenomenon is well established. This result also supported the hypothesis proposing that this pattern of membrane FA profile is closely related to obesity-induced risks for impaired insulin sensitivity [3,4].

Table 3. Body weight, body fat content, plasma lipids levels, and adipocyte membrane fatty acid composition in 8-weeks-old obese mice received two different diets for 4 weeks

	Low-fat (P/S=1.2, n=8)	High-Fat (P/S=0.6, n=8)
Body weight (g)	46.0±4.8	50.3±5.9*
Body fat content (%)	42.1±7.8	53.6±6.5*
Plasma lipid (mg/dl):		
TAG	130.8±19.3	188.5±20.7*
Total-CHOL	148.5±25.2	178.5±23.4
HDL	19.8±4.4	12.5±3.9*
Membrane FA (%):		
16:0 (Palmitic acid)	20.4±1.2	26.5±1.6*
18:0 (Stearic acid)	27.5±1.5	35.8±2.7*
18:1 (Oleic acid)	13.9±0.6	13.2±0.9
18:2 (Linoleic acid)	31.1±1.1	19.0±1.4*
20:4 (Arachidonic acid)	7.1±1.9	5.5±1.6
UI	104.5±5.5	73.2±4.3*
Footnotes are same as in Table 2.		

In this study, ob/ob mice fed a high-fat diet (low P/S ratio) had the highest body fat content. The intake of dietary fat, irrespective of its composition, is known closely associated with body weight gain and body fat accumulation [17]. Our data also showed the AD membrane of ob/ob mice would become more rigid when fed a high-fat diet. Increased SFA in AD membrane was mainly due to the increased corporation of these FA (from high-fat diet) into the membrane. Beef tallow, which was used in this study, is known to have much lower C18:2 and none C20:4 contents than swine lard and vegetable oils [18]. Thus, the effect of high-fat diet on decreasing AD membrane PUFA might be due to the less intake amount of C18:2 and its elongation to C20:4. Moreover, by comparing the UI values, our result showed that high-fat diet with low P/S ratio would additionally aggravate the AD membrane functions under their primarily genetic defects. Increased dietary saturated fats can decrease the basal metabolic rate via the reduced activity of membrane sodium pump (Na+/K+-ATPase), which accounts for a large fraction of energy consumption [19]. Adaptive thermogenesis exerted by uncoupled oxidation in brown AD mitochondria is also known to closely participate in the development of obesity and thus insulin resistance [20]. The synthesis of uncoupling proteins is mediated by peroxisome proliferator-activated receptors (PPAR), which can be regulated by FA [6]. Our data also showed the membrane fluidity (assessed by UI) of ob/ob mice was markedly improved by the diet with P/S=1.2 when compared to that of Purina chow (P/S=0.9) or high-fat diet (P/S=0.6). Although we did not measure the differences in insulin sensitivity between these diets in our ob/ob mice, our data were still able to support the suggestion that Mediterranean type diet is beneficial for alleviating the manifestations of type 2 diabetes mellitus [6-8].

In summary, the results of this study indicated that AD membrane FA composition was innately different and could be modified by dietary manipulation with various P/S ratios in ob/ob mice. Although the changes in AD membrane FA profile in ob/ob mice could be concluded deriving from its intrinsic obese-prone phenomenon, the pathophysiological mechanisms are still obscure. Further investigation into the involvement of hormonal factors and dietary manipulation regarding the alterations in AD membrane FA composition, and their roles in mediating intracellular signaling might throw lights on this subject.

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Declaration of interest

No conflict of interest.

References

[1]. M. M. Finucane, G. A. Stevens, M. J. Cowan, et al, National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants, *Lancet, 377,* 2011, 557-567.

[2]. P. E. Scherer, Adipose tissue: from lipid storage compartment to endocrine organ, *Diabetes, 55,* 2006, 1537-1545.

[3]. J. D. McGarry, Dysregulation of fatty acid metabolism in the etiology of type 2 diabetes, *Diabetes, 51,* 2002, 7-18.

[4]. C. Rattarasarn, Physiological and pathophysiological regulation of regional adipose tissue in the development of insulin resistance and type 2 diabetes, *Acta Physiol, 186,* 2006, 87-101.

[5]. M. T. Clandinin, S. Cheema, C. J. Field, M. L. Garg, J. Venkatraman, T. R. Clandinin, Dietary fat: exogenous determination of membrane structure and cell function, *FASEB J, 5,* 1991, 2761-2769.

[6]. M. Hagg, N. G. Dippenaar, Dietary fats, fatty acids and insulin resistance: short review of a multifaceted connection, *Med Sci Monit, 11,* 2005, RA359-367.

[7]. S. Phinney, Arachidonic acid maldistribution in obesity. *Lipids, 31,* 1996, S271-S274.
[8]. A. J. Hullbert, N. Turner, L. H. Storlien, P. L. Else, Dietary fats and membrane function: implications for metabolism and disease, *Biol Rev Camb Philos Soc, 80,* 2005, 155-169.
[9]. A. S. H. Costa, P. A. Lopes, M. Estevao, et al, Contrasting cellularity and fatty acid composition in fat depots from Alentejana and Barrosa bovine breed fed high and low forage diets, *Int J Biol Sci, 8,* 2012, 214-227.
[10]. J. Speakman, C. Hambly, S. Mitchell, E. Krol, The contribution of animal models to the study of obesity, *Lab Anima, 42,* 2008, 413-432.
[11]. P. G. Reeves, F. H. Nielsen, G. C. Fahey, AIN-93 purified diets for laboratory rodents, *J Nutr, 123,* 1993, 1939-1951.
[12]. P. Y. Lin, W. H. Lin, C. T. Tsou, Y. M. Song, M. D. Chen, Effect of zinc on cellular levels of calmodulin and cyclic adenosine monophosphate in the adipocyte. *Biol Trace Elem Res, 76,* 2000, 229-234.
[13]. C. J. Field, E. A. Ryan, A. B. R. Thomson, M. T. Clandinin, Diet fat composition alters membrane phospholipids composition, insulin binding, and glucose metabolism in adipocytes from control and diabetic animals, *J Biol Chem, 265,* 1990, 11143-11150.
[14]. Z. Li, T. Gu, B. Kelder, J. J. Kopchick, Analysis of fatty acids in mouse cells using reversed-phase high-performance liquid chromatography, *Chromatographia, 54,* 2001, 463-467.
[15]. A. Ibrahim, S. Natarajan, Ghafoorunissa, Dietary trans-fatty acids alter adipocyte plasma membrane fatty acid composition and insulin sensitivity in rats, *Metabolism,54,* 2005, 240-246.
[16]. S. M. Turpin, J. G. Ryall, R. Southgate, et al, Examination of 'lipotoxicity' in skeletal muscle of high-fat fed and ob/ob mice, *J Physiol, 587,* 2009, 1593-1605.
[17]. H. Takeuchi, T. Matsuo, K. Tokuyama, Y. Shimomura, M. Suzuki, Diet-induced thermogenesis is lower in rats fed a lard diet than those fed a high oleic acid safflower oil diet, a safflower oil diet, or a linseed oil diet, *J Nutr, 125,* 1995, 920-925.
[18]. J. D. Wood, M. Enser, A. V. Fisher, et al, Fat deposition, fatty acid composition and meat quality: a review, *Meat Sci, 78,* 2008, 343-358.
[19]. B. J. Wu, P. L. Else, L. H. Storlien, A. J. Hulbert, Molecular activity of Na+/K+-ATPase from different sources is related to the packing of membrane lipids, *J Exp Biol, 204,* 2001, 4271-4280.
[20]. J. Kopecky, P. Flachs, K. Bardova, P. Brauner, T. Prazak, J. Sponarova, Modulation of lipid metabolism by energy status of adipocytes: implications for insulin sensitivity, *Ann NY Acad Sci, 967,* 2002, 88-101.