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The Effects of Fatty Acid Composition on Cardiac Hypertrophy and Function in Mouse Models of Diet-Induced Obesity

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Abstract

High fat diets (HFDs) are used frequently to study the development of cardiac dysfunction in animal models of obesity and diabetes. However, impairment in systolic function, often reported as declining ejection fraction, may not consistently occur in a given time frame which could be contributable to a variety of factors within the experimental design. One major factor may be the amounts of saturated and unsaturated fatty acids (FAs) that are present in the diet. To determine whether the FA content and composition was a critical determinant in the development of cardiac dysfunction in response to high fat feeding, we fed adult, male mice either Western diet (WD, 45% fat, 60% saturated), Surwit diet (SD, 60% fat, 90% saturated), milk-fat based diet (MFBD, 60% fat, 60% saturated), or high fat Western diet (HFWD, 60% fat, 32% saturated) for 12 weeks. We report that neither the amount of total fat nor the ratio of saturated to unsaturated FAs in the diets differentially affect body weight and adiposity in mice. In addition, no evidence of systolic dysfunction is present after 12 weeks. Interestingly, the HFWD, with equal parts saturated, monounsaturated, and polyunsaturated FAs, induces mild cardiac hypertrophy and diastolic dysfunction after 12 weeks, which coincides with elevated serum levels of arachidonic acid. Our results suggest that the dietary FA content and composition may be a primary determinant of diastolic, but not systolic, dysfunction in animal models of diet-induced obesity.

Keywords

lipid overload; lipid metabolism; cardiomyopathy; fatty acid profiling; mouse strain

1. INTRODUCTION

High fat diets (HFDs) are used extensively in animal models to investigate the contribution of diet-induced obesity and Type II diabetes to the development of cardiac dysfunction.

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Although HFDs produce quite rapid and predictable obese and diabetic phenotypes in rodents, the time course for the incidence of cardiac dysfunction, reported as reduced ejection fraction (i.e., systolic dysfunction) is variable, spanning from 4 weeks [1], to 12–16 weeks [2–4], or as long as 22 weeks [5]. In contrast, some reports find no evidence of systolic dysfunction after 6 or 16 months of HFD [6–8]. Many factors may explain the discrepancies reported in the literature including: the mouse strain, sex, and the age of the mice when the diet was initiated. However, the specific composition of the diet, including the total calories from fat and the ratio of saturated to unsaturated fats, is often overlooked and may be a plausible explanation for the reported variability.

The concept that dietary consumption of unsaturated fatty acids (UFAs) or saturated fatty acids (SFAs) influence cardiac function is supported by epidemiological data as diets high in the consumption of UFAs are associated with decreased risk in the development of cardiovascular disease [9]. In fact, both the American Heart Association and the American College of Cardiology encourage the replacement of SFAs with UFAs in their dietary guidelines [10]. Basic science has provided mechanistic insight into the negative cellular effects of SFAs, particularly palmitate and stearate, supporting the concept that SFAs are toxic to normal cellular processes [11, 12]. In this regard, diets high in SFAs may likely induce cardiac dysfunction in a more rapid manner than diets high in UFAs, thus confirming the importance of dietary FA composition in animal models of diet-induced obesity.

To determine whether FA content and/or composition are important factors for cardiac remodeling in diet-induced obesity, we used four HFDs that differed in total calories from fat as well as having distinct FA profiles. Our data shows that the dietary FA composition does not differentially affect the development of obesity or induce systolic cardiac dysfunction after 12 weeks of feeding. However, a HFD that provides equal parts of SFAs, monounsaturated FAs (MUFAs), and polyunsaturated FAs (PUFAs) surprisingly induces both cardiac hypertrophy and diastolic dysfunction in mice. The impaired diastolic function in mice was accompanied by changes in serum lipids, including increased abundance of arachidonic acid. Overall, our findings suggest that dietary FA composition may be a primary determinant of cardiac hypertrophy and diastolic dysfunction, but not obesity or systolic dysfunction, in HFD mouse models.

2. METHODS

2.1. Animal Models

Male, FVB/N (Charles River, Wilmington, MA) and C57BL/6J (Jackson Labs, Bar Harbor, ME) mice at 10–12 weeks of age were used. Studies were approved by the University of Washington Institutional Animal Care and Use Committee. Mice were kept on a 12-hour light/dark cycle with water and food ad libitum.

2.2. High Fat Diets

Chow fed mice received PicoLab Rodent Diet 20 (#5053, LabDiet, St. Louis, MO). The Western Diet (WD, #D12079B), Surwit Diet (SD, #D12331), and High Fat Western Diet (HFWD, #D12492) were purchased from Research Diets (New Brunswick, NJ). The Milk

Fat Based Diet (MFBD, #TD.09766) was purchased from Envigo Teklad Diets (Madison, WI). High fat diets supplied to the mice were changed every two days during the 12 week feeding study.

2.3. Serum and Tissue Harvest

At the completion of the 12 week feeding period, mice were fasted for 3 hours prior to euthanasia with an I.P. injection of sodium pentobarbital. Blood glucose was measured from a tail clip via the Contour blood glucose monitoring system (Bayer Healthcare, Mishawaka, IN). After thoracotomy, ~500µl of blood was obtained from the left ventricle via cardiac puncture. Hearts were dissected from the thoracic cavity and quickly rinsed in ice cold phosphate buffered saline (PBS). Excess tissue was trimmed, the heart gently blotted dry on gauze pads, and weighed on a balance (Mettler Toledo, Columbus, OH). To determine tibia length (TL), the right tibia of each mouse was dissected, cleaned of tissue, and measured with a Vernier caliper. Heart weight (HW) was normalized to TL to assess cardiac hypertrophy. The epididymal and retroperitoneal fat pads were dissected, gently blotted, and weighed. The sum of these values was used to estimate adiposity.

2.4. Serum Analysis

Non-esterified fatty acids (NEFA), triglycerides, and cholesterol were measured in mouse serum using commercially available assays (Wako Chemicals, Richmond, VA).

2.5. Lipid Extraction

FA composition was assessed in lipid extracts from food pellets and mouse serum. In brief, 50mg of food pellets or 100µl of mouse serum was homogenized in 2:1 chloroform:methanol. After centrifugation, the chloroform layer was collected, dried under a nitrogen stream, and resuspended in 2.5% sulfuric acid:methanol. The solution was incubated at 80°C for 60 minutes to create fatty acid methyl esters (FAMES). The solution was then rinsed twice with 500µl of hexane. The resultant hexane was dried under nitrogen stream and resuspended in 50µl of hexane.

2.6. Gas chromatography-mass spectrometry (GC-MS) Analysis

Gas chromatography (GC) was performed on an Agilent 7890B system using a DB-225MS column. The injector was set at 240°C using splitless injection mode, and 1 µL injections were made. The temperature gradient started with an initial temperature of 100°C, followed by a linear increase to 180°C at 20°C/min, and a slower linear increase to 220°C at 5°C/min. Total run time was 25 minutes. The Agilent 5977A mass spectral detector was operated in scan mode at 275°C. Individual FA species were confirmed by the use of the Supelco® 37 Component FAMES mix (Sigma-Aldrich, St. Louis, MO). Peak areas of each FA were measured by integration of the chromatogram using the MassHunter software (Agilent, Santa Clara, CA). The relative abundance was determined by the ratio of each FA to the total of all FAs identified in the sample.

2.7. Echocardiography

Trans-thoracic echocardiography was conducted in anesthetized mice under 1% isoflurane at the end of 12 weeks of HFD. M-mode image sequences and Tissue Doppler imaging (TDI) was performed on the Vevo 2100 High-Resolution Imaging System (VisualSonics, Toronto, Ontario, Canada) with a MS400 MicroScan Transducer, centered at 30 MHz [13, 14].

2.8. Statistical Analysis

All values are reported as mean $\pm$ standard error of the mean (SEM). For multiple group comparison, the one way analysis of variance (ANOVA) followed by a Bonferroni post hoc analysis was used. For time course comparisons, a repeated measures ANOVA followed by a Bonferroni post hoc analysis was used. Statistical significance was tested at the $P < 0.05$ level.

3. RESULTS

3.1. Macronutrient and fatty acid profiles of diets used in the study

Using information provided by each company, we compared the macronutrient profile of each HFD to standard chow. In Figures 1A–1E, the relative percentages of total calories from carbohydrates (CHO), saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and protein are presented. The Western Diet (WD) contained 45% of the total calories from fat, with 60% of those calories arising from SFAs (Figure 1B). The Surwit Diet (SD), the Milk-fat Based Diet (MFBD), and High Fat Western Diet (HFWD) all contained 60% of the total calories from fat, with varying percentages of SFAs, MUFAs, and PUFAs (Figures 1C–1E). Of note, the SD contained 90% of the total calories of fat in the form of SFA (Figure 1C), while the HFWD contained equal parts SFA, MUFA, and PUFA (Figure 1E). Despite varying percentages of fat in the four HFDs, the percent total calories from protein were similar.

To gain further insight into the FA composition of each diet, we performed gas chromatography-mass spectrometry (GC-MS) analysis on lipid extracts from the food pellets to determine the relative abundance of each fatty acid. Although palmitate (C16:0), stearate (C18:0), and oleate (C18:1) were generally predominant in all diets, some notable differences were observed. Linoleate (C18:2) was in high abundance in both the chow (Figure 1F) and HFWD (Figure 1J) but minimally present in the remaining diets. The WD and MFBD were similar in the FA composition (Figures 1G, 1I). The SD was characterized by a high abundance of medium chain SFAs, particularly laurate (C12:0) as well as the long chain SFA, myristate (C14:0, Figure 1H). The HFWD was primarily limited to four FAs: palmitate (C16:0), stearate (C18:0), oleate (C18:1), and linoleate (C18:2) as shown in Figure 1J.

3.2. Changes in the serum lipid profile in response to high fat feeding

Elevations in serum lipids are expected in models of diet-induced obesity. Therefore, we performed biochemical assays on serum collected from chow and HFD fed mice. The WD caused robust increases in serum concentrations of NEFAs and total cholesterol, but not triglycerides, whereas NEFAs, triglycerides, and total cholesterol were not significantly

increased by the SD in FVB/N relative to chow-fed mice (Figures 2A–2C). In C57BL6/J mice, both MFBD and HFWD increased serum NEFA levels without increasing serum triglycerides (Figures 2D, 2E). Total cholesterol in the serum was significantly increased only in mice fed the HFWD (Figure 2F).

We performed additional analysis of serum lipid extracts using GC-MS with the expectation that the serum FA composition would closely resemble the dietary FA composition. Surprisingly, the FA composition of the diet (Figure 1F–1J) was not consistent with the FA composition of the serum (Figures 2G, 2H). For example, despite elevated exogenous supply of palmitate (C16:0) from the WD and MFBD, the relative abundance in the serum was similar to chow (Figures 2G, 2H). Laurate (C12:0) represented ~40% of the FAs supplied by the SD; however, it represented only 1% of the total in serum lipid extracts (data not shown). The HFWD provided high abundance of oleate (C18:1) and linoleate (C18:2) but both of these fatty acids were reduced compared to chow. Of interest was the significant increase in arachidonic acid (C20:4) in the serum from the SD, MFBD, and HFWD fed mice despite low to no availability in the diet. All told, these data demonstrate that the availability of fatty acids found in the serum does not directly reflect the FAs of diets and suggests that differential partitioning of FA occurs in the peripheral tissues.

3.3. High fat diet effects on body weight and adiposity

To evaluate whether the content or composition of dietary fat differentially affected obesity, we obtained body weights and adipose tissue mass from the mouse cohorts. In the FVB/N strain, body weight increased similarly with both diets over the 12-week period with an approximate 2-fold increase in adipose mass (Figures 3A, 3B). In the C57BL6/J strain, both the MFBD and HFWD also significantly increased body weight and adiposity after 12 weeks of feeding (Figures 3D, 3E). Although the HFWD appeared to increase body weight to a greater degree, the relative change from baseline was not statistically different than MFBD (Figure 3D). Strikingly, the relative change in both body weight and adipose tissue mass was greater in the C57BL6/J cohorts compared to the FVB/N cohorts (Figures 3A, 3B vs. Figures 3D, 3E). To assess whether obesity in these groups was accompanied by diabetes, we measured serum glucose levels at the end of the 12 weeks. Neither the WD nor the SD led to hyperglycemia in FVB/N mice (Figure 3C). However, the HFWD, but not the MFBD, led to significant increases in serum glucose levels (Figure 3F). These data suggest that the FA composition of the diet does not differentially affect body weight gain or adiposity in mice fed a HFD for up to 12 weeks.

3.4. The effect of high fat diets on cardiac function and hypertrophy

We performed in-vivo assessment of systolic cardiac function via echocardiography in all cohorts at the end of 12 weeks. At similar heart rates (Figures 4A, 4C), none of the diets caused a significant decrease in fractional shortening (Figures 4B, 4E). Furthermore, the WD and SD did not induce cardiac hypertrophy, assessed by the heart weight to tibia length ratio (HW:TL) in FVB/N mice (Figure 4C). However, the MFBD and HFWD caused an approximate 15% and 10% increase in HW:TL, respectively, in the C57BL6/J strain (Figure 3F). Based on the hypertrophy effect in the C57BL6/J strain, we assessed diastolic function using Tissue Doppler Imaging. As shown in Figures 3G–3I, the HFWD caused a significant

decrease in the E'/A' ratio as well as a significant increase in the isovolumic relaxation time (IVRT), suggestive of diastolic dysfunction. These data suggest that 12 weeks of high fat feeding, regardless of the dietary FA content or composition, does not induce systolic dysfunction in FVB/N or C57BL6/J. However, the dietary FA content and composition may influence the development of cardiac hypertrophy and diastolic dysfunction in obese, C57BL6/J mice.

4. DISCUSSION

In the present study, we analyzed the effects of various HFDs on the development of obesity, cardiac dysfunction, and serum lipid profiles. We report that body weight and adiposity increases similarly despite varying percentages of total fat and ratios of SFA, MUFA, and PUFA, with the caveat that C57BL6/J mice increase body weight and adiposity to a higher degree than FVB/N. In addition, neither strain develops systolic dysfunction after 12 weeks of high fat feeding. However, the C57BL6/J strain develops mild cardiac hypertrophy and, if fed a diet with equal parts of SFA, MUFA, and PUFA, displays evidence of diastolic dysfunction. Although anticipated changes in some serum lipid concentrations were observed, the composition of individual FAs in the serum was not consistent with the dietary supply. Overall, these findings suggest the dietary fat content or composition is not a factor in the development of obesity or systolic dysfunction in mice fed a HFD for three months. However, the FA composition of the diet may be critical in the pathogenesis of cardiac hypertrophy and diastolic dysfunction although further investigation is necessary.

One major finding of the present study is the failure of HFDs to produce evidence of systolic dysfunction after 12 weeks. In the FVB/N strain, the lower increase in body weight and adiposity relative to the C57BL/6J strain could be a likely explanation. However, the lack of systolic dysfunction, despite dramatic increases in body weight and adiposity, in C57BL/6J mice is disappointing as several studies have reported significant systolic dysfunction in the 12–16 week time frame [2–4]. Our study suggests that the discrepancies do not appear to be specific to the ratio of SFAs to UFAs in the diet and that other dietary components such as sucrose, fructose, or other micronutrients require further examination.

Another important finding of the present study is the strain-specific differences in the development of obesity and cardiac hypertrophy in response to various HFDs. The C57BL/6J strain is well-documented to be particularly useful in diet-induced obesity models [15, 16]. However, bioengineered mouse models, particularly transgenic overexpression, are often initiated on the FVB/N mouse strain. The data presented herein would encourage researchers to backcross the transgenic line to the C57BL6/J strain before initiation of a HFD study, particularly if investigating cardiac remodeling in obesity. The increase in cardiac hypertrophy observed with the MFBD has been previously shown and linked to elevations in C14:0-ceramides in cardiac tissue [3]. Although ceramides were not assessed in this study, our data would support the MFBD as a diet that induces cardiac hypertrophy associated with obesity.

The observations of obesity, mild cardiac hypertrophy, and diastolic dysfunction after 12 weeks would support using the HFWD, or a similar diet with relatively equal parts of SFA,

MUFA, and PUFA, for studies assessing cardiac remodeling during the development of obesity. The more robust phenotype could be due to the increased abundance of arachidonic acid (AA) in the serum. AA is an omega-6 FA that can be converted from linoleate (C18:2), which was in abundant supply in the HFWD. AA can also be metabolized to eicosanoids which can activate both pro- and anti-inflammatory pathways. Whether the increased AA is directly responsible for the observed diastolic dysfunction was not directly tested in our study and needs additional examination. Interestingly, Omega-6 FAs are suggested to be cardioprotective although the dietary Omega-3 to Omega-6 FA ratio may be more pertinent as low eicosapentaenoic acid (EPA) to AA ratio was indicated as an independent risk factor of cardiac mortality in heart failure patients [17, 18].

The expectation that diets high in SFAs would result in impaired cardiac function after 12 weeks was not supported from our present study. Previous studies in cell culture models demonstrated that the SFAs, palmitate and stearate, are toxic to cells while supplementing the MUFA, oleate, alleviates the lipotoxic effect [19, 20]. In this regard, the lack of systolic dysfunction in the WD and MFBD mice could be due to the high abundance of oleate in the diet. Another explanation may exist. In the WD serum, no increase in the SFAs, palmitate and stearate, was observed; however, the abundance of the MUFAs, palmitoleate and oleate, was increased significantly. Delta-9 desaturases are known to convert SFAs into MUFAs and the increased abundance of MUFAs may signify an upregulation of desaturase activity in peripheral tissues that resists the development of SFA-induced lipotoxicity. Furthermore, previous studies in rats and humans demonstrated that FAs with a lower carbon number and degree of unsaturation are more readily oxidized, with laurate (C12:0) more readily oxidized than other predominant FAs in serum [21, 22]. This preferential oxidation of laurate in peripheral tissues could explain the relatively low abundance (~1%) in the mice fed the SD compared to the high dietary abundance (~40%). Therefore, profiling the FA abundance in the serum and peripheral tissues may be a useful tool to facilitate understanding of lipid partitioning and other aspects of lipid metabolism in diseased models.

In summary, we assessed the development of obesity, cardiac hypertrophy, and cardiac dysfunction on various HFDs in both FVB/N and C57BL/6J mice. Our findings demonstrate that obesity and cardiac hypertrophy is more pronounced in C57BL/6J mice after 12 weeks of HFD. Despite variations in both total fat and saturated fat in the diets, systolic function is well maintained in both mouse strains after 12 weeks of feeding. However, evidence of diastolic dysfunction was observed in a HFD with equal ratios of SFA, MUFA, and PUFA which coincided with elevated serum levels of AA.

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Figure 1. Profile of high fat diets used in mouse studies
A–E) Relative amounts of carbohydrates (CHO), saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and protein of chow and various high fat diets used in the study are presented based on information provided by the manufacturer. F–J) The relative abundance of fatty acids analyzed in lipid extracts from food pellets. Fatty acids are organized by degree of unsaturation and carbon length (n=3).

Figure 2. Changes in the serum lipid profile in response to high fat feeding

A) Non-esterified fatty acids; B) triglycerides; and C) total cholesterol measured in serum from FVB/N mice fed chow, Western Diet (WD), or Surwit Diet (SD) for 12 weeks (n=6–8).

D) Non-esterified fatty acids; E) triglycerides; and F) total cholesterol measured in serum from C57BL6/J mice fed chow, milk fat based diet (MF) or high fat western diet (HW) for 12 weeks (n=5–9).

G) Fatty acid abundance of whole serum lipid extracts from HFD fed FVB/N mice analyzed via GC-MS (n=4).

H) Fatty acid abundance in whole serum lipid extracts from HFD fed C57BL6/J mice analyzed via GC-MS (n=4–5). * P<0.05 vs chow. # P<0.05 vs WD. # P<0.05 vs MFBD.

Figure 3. High fat diet effects on body weight and adiposity

A) Weekly body weight obtained in FVB/N mice fed chow, Western Diet (WD), or Surwit Diet (SD) for 12 weeks (n=8–14). B) Adipose tissue mass (sum of epididymal and retroperitoneal fat pads) harvested from mice after 12 weeks of HFD (n=6–9). C) Blood glucose measured in mice after 3 hours of food withdrawal (n=6–9). D) Weekly body weight (n=12–20); E) adipose tissue mass (5–9); and F) blood glucose (n=5–10) in C57BL6/J mice fed chow, milk fat based diet (MF) or high fat western diet (HW). * P<0.05 vs chow.

Figure 4. The effect of high fat diets on cardiac function and hypertrophy

A) Heart rate and B) fractional shortening measured by echocardiography in FVB/N mice fed chow, Western Diet (WD), or Surwit Diet (SD) for 12 weeks (n=5–9). C) Cardiac hypertrophy, assessed by the heart weight to tibia length ratio (HW:TL) after 12 weeks of HFD (n=5). D) Heart rate and E) fractional shortening measured by echocardiography in C57BL/6J mice fed chow, milk fat based diet (MF) or high fat western diet (HW) for 12 weeks (n=6–15). F) HW:TL (n=6–15). G–I) Measures of diastolic function, E/E' and E'/A' ratios and isovolumic relaxation time (IVRT), measured by Tissue Doppler Imaging (n=6–10). * P<0.05 vs chow.