Effect of Male Sex and Obesity on Platelet Arachidonic Acid in Spontaneous Hypertensive Heart Failure Rats

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Sexual dimorphism is observed in the progression to congestive heart failure and, ultimately, in longevity in spontaneously hypertensive heart failure (SHHF) rats. As platelet activation may impact development of cardiovascular diseases, we studied the effects of obesity and sex on platelet polyunsaturated fatty acid (PUFA) profile and its relationship to platelet aggregation in 6-month-old SHHF rats. After a 24-hr fast, blood was obtained for measurement of platelet phospholipid omega-6 (n-6) and omega-3 (n-3) PUFA. Collagen-induced platelet aggregation was measured by whole-blood impedance aggregometry. Obese male (OM) SHHF had significantly more platelet arachidonic acid (AA) and total n-6 PUFA than lean males (LMs), lean females (LFs), or obese females (OFs). Platelet aggregation was enhanced in males compared to females, with OMs by 45% compared to OFs and with LMs by 28% compared to LFs. Though no difference was found between OFs and LFs, platelet aggregation was increased in OMs by 20% compared to LMs. Though not significantly different, lag time to initiate platelet aggregation tended to be shortest in OMs and then, in increasing duration, LMs, LFs, and OFs, suggesting that platelets from male rats were quicker to aggregate than those from females. Platelet aggregation was correlated with platelet AA and total n-6 PUFA content. There was no relationship between n-3 PUFA and platelet aggregation. In SHHF rats, elevated AA and n-6 PUFA levels in platelets are associated with enhanced platelet aggregation. This relationship is potentiated by obesity and male sex. Exp Biol Med 229:657–664, 2004

Key words: obesity; male; platelet; arachidonic acid; SHHF rats

Introduction

A relationship between n-6 polyunsaturated fatty acid (PUFA) metabolism and the development of hypertension, diabetes, and cardiovascular disease has been suggested (1–5). As a member of the n-6 PUFAs, arachidonic acid (AA) is a precursor of thromboxane A_2 (TXA_2), a potent promoter of platelet aggregation and a vasoconstrictor (6–8). Alterations in n-6 PUFA metabolism and subsequent increases in AA production can contribute to platelet hyper-reactivity and aggregation by providing increased substrate for the production of TXA_2 (6–8). This may result in a state of dynamic vasoconstriction and can exacerbate the transition to heart failure (8–12). Risk factors that are associated with alterations in PUFA metabolism, increases in AA production, and activation of platelets include obesity, diabetes, and aging (10, 11, 14–21).

The spontaneous hypertensive heart failure (SHHF/Mcc-fa^{cp}; abbreviated SHHF) rat is a genetic model of hypertension and congestive heart failure (CHF). Though all SHHF rats develop hypertension at a similar age, the age at which transition to heart failure occurs in this rat strain is modulated by sex, leptin resistance, and obesity (22–25). SHHF rats that are homozygous for the corpulent (fa^{cp}) gene are leptin resistant due to failure to produce a functional leptin receptor (26, 27). Consequently, they become markedly obese, secondary to leptin resistance (26, 28, 29). Obese SHHF rats exhibit anomalies similar to human Syndrome X, such as hyperinsulinemia, impaired glucose tolerance, hyperlipidemia, and hypertension (29–31). In addition, this rat model of leptin resistance and obesity shows alterations in neurohumoral activation that may modulate blood pressure control and progression to CHF (25, 30, 32, 33). It is well documented that both male and female obese SHHF rats die of CHF at a younger age than their lean counterparts (+/+ or +/fa^{cp}; Refs. 28, 29, 31).

Progression of cardiovascular disease in the SHHF rat is also modulated by sex. Male SHHF rats develop significant cardiac and left ventricular hypertrophy and die

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from CHF at an earlier age compared with female rats (22, 25, 28, 31). Significant decline in cardiac function occurs in female SHHF rats after they cease to cycle, when serum estrogen concentrations begin to fall (34). These observations indicate that female sex may delay the onset or progression of heart failure in SHHF rats. In humans, onset of cardiovascular disease in women is also frequently delayed until after menopause, although the mechanism underlying this effect of sex hormones still remains controversial (35).

Although many studies have been conducted to understand the pathogenesis and mechanisms of metabolic syndromes that may contribute to the progression of cardiovascular disease in the SHHF rat, PUFA profile and its influence on platelet aggregation has not been assessed. Therefore, we examined the effect of obesity and sex on n-6 and n-3 PUFA profile in platelets and its relationship to platelet aggregation in this rat model of hypertension and CHF.

Materials and Methods

Animals and Diets. Obese male (OM; fa^{cp}/fa^{cp}, n = 5), obese female (OF; fa^{cp}/fa^{cp}, n = 5), lean male (LM; +/?, n = 5), and lean female (LF; +/?, n = 6) SHHF rats were obtained from the colony maintained by Dr. Sylvia McCune at The Ohio State University. Six-month-old rats were studied because, at this age, SHHF rats have established hypertension, but cardiac decompensation has not yet occurred (25, 36). All experimental procedures were approved by and conformed to the standard of the University Institutional Laboratory Animal Care and Use Committee. Animals were fed commercial rat chow (Agway PROLAB Rat/Mouse/Hamster 3000; Purina Mills, Inc., New Albany, IN) and water *ad libitum* until 6 months of age.

Body, Brain, and Heart Weights, Blood Pressure Measurement, and Blood Collection. After a 24-hr fast; body weight and systolic pressure were measured. The tail cuff method (Model ICT-2H, Gilson Duograph, Middleton, WI) was used to measure systolic blood pressure. Between 0900–1000 hrs, rats were anesthetized by intraperitoneal injection of 100 mg/kg pentobarbital, and blood was collected directly by cardiac puncture. Six milliliters of blood was immediately transferred into a tube containing 3.8% sodium citrate solution. Remaining blood was allowed to coagulate, and serum was collected for later determination of triglycerides and cholesterol. Heart and brain were weighed, and heart to brain weight ratios were calculated. Heart/brain weight ratios are routinely used to assess the extent of cardiac hypertrophy in SHHF rats because the severe obesity makes heart/body weight ratios meaningless (37). Brain weight reflects lean body mass, is easy to obtain, and is reproducible.

Blood Lipid Measurement. Triglycerides were measured with an enzymatic triglyceride Test Set GPO-PAP Method (Stanbio Lab., Houston/San Antonio, TX). Cholesterol was determined by enzymatic method using Direct Cholesterol Test Set (Stanbio Lab.).

Hematology and Platelet Phospholipid Fatty Acid Measurement. Platelet count, mean platelet volume (MPV), and hematocrit were determined from 0.5 ml of citrated blood using an automated cell counter (Coulter S-Plus 4; Coulter Electronics, Hialeah, FL). Platelets were harvested as described by Kwon *et al.* (38). Citrated blood was centrifuged at 200 *g* for 10 mins at 4°C to obtain platelet-rich plasma. The platelet-rich plasma was recentrifuged at 5000 *g* for 10 mins at 4°C to pellet platelets. Five milliliters of ice-cold Tris-HCl (154 m*M*, pH 7.5)/NaCl (0.9%)/EDTA (77 m*M*) buffer was used to wash platelets after the supernatant containing platelet-poor plasma was discarded. Following centrifugation at 1000 *g* for 10 mins at 4°C, platelet pellets were resuspended in 1 ml of saline solution. Lipids were extracted according to the method of Folch *et al.* (39).

Phospholipid fractions were separated by Silica gel G thin-layer chromatography (TLC) plates (1000 μm; Analtech, Newark, DE) using the method with *n*-hexane:diethyl ether:glacial acetic acid (80:20:1, v/v/v) as the developing solvent described by Christie (40). Visualization of lipid spots was performed according to Nakamura *et al.* (41). The spot containing phospholipids was scraped off and collected in culture tubes, which were then flushed with N_2 and stored at –20°C until the next procedures. Methylation of fatty acids was based on the method of Morrison and Smith (42) except that borontrichloride methanol was used as the methylation reagent. The fatty acid methyl esters were transferred to a 4-ml glass vial with a Teflon-coated screw cap, flushed with N_2 gas, wrapped with parafilm, and stored at –20°C until gas chromatography (GC) analysis.

Fatty acid profiles were analyzed using gas chromatography (Hewlett Packard, 5890A, Series II) on a capillary column (J&W Scientific, Palo Alto, CA, DB-23, 30 m x 0.53 mm i.d. x 0.5 μm, Varian, San Fernando, CA) with a flame ionization detector. Injector temperature was 220°C, and detector temperature was controlled at 230°C. The temperature program began at 125°C, increased 10°C per min, held at 175°C for 5 mins, increased at the rate of 5°C per min, and held at 210°C for 4 mins. Fatty acid standards (Matreya, Inc., Pleasant Gap, PA) were used to determine peaks for each fatty acid. Fatty acid distribution was calculated as wt% (micrograms of each fatty acid/micrograms of total fatty acids of platelet phospholipids x 100%). Total n-6 PUFA were calculated as the sum of linoleic (LA: 18:2n6), γ-linolenic (GLA: 18:3n6), dihomo-γ-linolenic (DGLA: 20:3n6), and arachidonic (AA: 20:4n6) acids. Total n-3 PUFA was calculated as the sum of α-linolenic (ALA: 18:3n3), eicosapentaenoic (EPA: 20:5n3), and docosahexaenoic (DHA: 22:6n3) acids.

Platelet Aggregation. Platelet aggregation was measured using the Chronolog Model 500 Whole Blood Lumi Aggregometer (Chronolog Corp., Havertown, PA).

Table 1. Body Weights, Heart Weights, and Serum Biochemical Parameters in SHHF Rats[a]

	Obese SHHF		Lean SHHF		Two-factor ANOVA *P* value		
	Male	Female	Male	Female	Sex	Obesity	Sex × obesity
Body weight (g)	585.6 ± 21.5[bc]	466.0 ± 8.8[c]	398.8 ± 6.2[b]	220.2 ± 5.8	<0.001	<0.001	0.024
Heart weight (g)	1.73 ± 0.07[b]	1.50 ± 0.05[c]	1.69 ± 0.08[b]	1.11 ± 0.02	<0.001	0.001	0.006
Brain weight (g)	1.79 ± 0.03	1.79 ± 0.04	1.90 ± 0.04[b]	1.74 ± 0.03	0.031	0.351	0.039
Heart weight/brain weight (ratio)	0.97 ± 0.04	0.84 ± 0.03[c]	0.89 ± 0.04[b]	0.64 ± 0.02	<0.001	<0.001	0.081
Systolic blood pressure (mm Hg)	174.6 ± 3.2	175.7 ± 2.9	183.3 ± 3.4	180.2 ± 2.4	0.756	0.055	0.522
Fasted plasma triglycerides (mg/dl)	774 ± 133[c]	879 ± 76[c]	39 ± 11	54 ± 2	0.472	<0.001	0.592
Fasted plasma cholesterol (mg/dl)	138 ± 12[bc]	68 ± 20	48 ± 10	54 ± 18	0.004	<0.001	0.001

[a] All values are mean ± SEM. SHHF, spontaneous hypertension heart failure; ANOVA, analysis of variance.
[b] Obese male is significantly different from obese female, or lean male is significantly different from lean female, $P < 0.05$.
[c] Obese is significantly different from lean of the same sex, $P < 0.05$.

For aggregation, 0.55 ml of 0.9% fresh saline was added to 0.45 ml of citrated whole blood in a plastic cuvette that contained a siliconized stir bar. Before the addition of agonist, cuvettes containing the diluted blood samples were placed into a heater block, warmed to 37°C, and stirred at 1000 rpm to achieve temperature equilibrium. After a stable baseline was achieved, 4 μg/ml of collagen was added to induce platelet aggregation. Impedance, as an indicator of platelet aggregation, was recorded for 6 mins. Aggregation was expressed as the increase in impedance measured between two electrodes on which platelet aggregation occurred. The change in impedance was compared to that of a 20-ohm internal standard. Centimeters of deflection from baseline were converted to ohms. Lag time was calculated as the time interval in minutes from the time collagen was added until aggregation began.

Statistical Analysis. Data are expressed as mean ± standard error mean (SEM). Two-factor analysis of variance to assess the effect of sex and obesity and Student's *t* tests were used where appropriate. Means between groups were considered significantly different at $P < 0.05$. Linear regression analysis was used to assess the relationship between platelet parameters and PUFAs.

Results

Weights and Blood Pressure. Body weight was greatest in OMs and then, in descending order, OFs, LMs, and LFs, showing significant differences between obese and lean and between male and female groups (Table 1). Systolic blood pressure was not modulated by obesity or sex (Table 1). However, female sex was associated with a smaller heart weight in the lean animals (Table 1). This sex difference was lost when females were obese, and OFs had similar heart to brain weight ratios compared to OMs.

Blood Lipids. Both OMs and OFs showed significantly higher fasted serum triglyceride concentrations than LMs and LFs, respectively (Table 1). There was no difference in serum triglyceride due to sex. There was a synergist effect of obesity and male sex resulting in a significantly higher serum cholesterol concentration in the OMs.

Platelet Phospholipid Fatty Acids. There was no effect of sex on platelet phospholipid PUFA profile in lean SHHF (Table 2). Obesity was not associated with significant alteration in PUFA profile in females. There was an effect of obesity and sex resulting in significantly higher platelet AA and total n-6 PUFA content in OMs compared to either LMs or OFs (Table 2). In general, males had higher levels of total n-3 PUFA compared to females. Though not statistically different, the ratio of total n-6 to total n-3 PUFA was highest in OMs, which had nearly 4-fold greater n-6 compared to n-3. Lean males had similar amounts of n-6 compared to n-3 PUFA, whereas OFs and LFs had higher n-6 compared to n-3. Both AA and total n-6 PUFA were correlated with serum cholesterol ($R = 0.76$ and $r = 0.74$, respectively, $P < 0.001$).

Hematology. Obesity may be associated with an increase in platelet numbers (Table 3), with OFs having significantly higher platelet counts compared to LFs. A similar trend was observed in the males, with OMs having higher platelet counts than LMs (OMs vs. LMs, $P = 0.0530$). Lean females also had smaller platelets (MPV) compared to LMs.

Platelet Aggregation. In general, males had enhanced platelet aggregation compared to females (Table 3). Platelet aggregation in OMs was increased by 20% compared to LMs and by 45% compared to OFs. Platelet aggregation in LMs was enhanced by 28% compared to LFs and by 21% compared to OFs. Although there were no significant differences, the lag time to initiation of platelet aggregation in response to collagen stimulation was shortest in OMs and then, in order of increasing duration, LMs, LFs, and OFs.

Correlations. Platelet aggregation and lag time showed a significant inverse relationship (Fig. 1). This

Table 2. Profile of Platelet Phospholipid Omega 6 and Omega 3 Polyunsaturated Fatty Acids in SHHF Rats[a]

	Obese SHHF		Lean SHHF		Two-factor ANOVA *P* value		
PUFA (wt%)	Male	Female	Male	Female	Sex	Obesity	Sex × obesity
LA (18:2n6)	4.53 ± 1.22	2.44 ± 0.60	1.85 ± 0.38	2.61 ± 0.51	0.377	0.105	0.069
GLA (18:3n6)	0.15 ± 0.09	0.23 ± 0.11	0.37 ± 0.18	0.11 ± 0.07	0.464	0.668	0.169
ALA (18:3n3)	0.41 ± 0.09[c]	0.45 ± 0.24	2.17 ± 0.69	1.39 ± 0.74	0.513	0.025	0.469
DGLA (20:3n6)	0.52 ± 0.14[b]	0.13 ± 0.07	0.20 ± 0.06	0.25 ± 0.08	0.080	0.305	0.030
AA (20:4n6)	14.04 ± 3.55[bc]	5.00 ± 1.73	5.02 ± 1.33	4.86 ± 1.52	0.048	0.049	0.056
EPA (20:5n3)	1.00 ± 0.13	0.44 ± 0.19	0.68 ± 0.46	0.48 ± 0.24	0.210	0.643	0.532
DHA (22:6n3)	5.83 ± 1.58	4.36 ± 0.42	5.33 ± 1.64	2.07 ± 0.68	0.074	0.276	0.480
Total n-6	19.23 ± 4.49[bc]	7.80 ± 2.11	7.44 ± 1.55	7.83 ± 1.73	0.054	0.042	0.041
Total n-3	7.23 ± 1.52	4.28 ± 1.11	8.18 ± 1.99	3.95 ± 0.70	0.017	0.828	0.646
Total n-6/total n-3 ratio	3.73 ± 1.39	1.73 ± 0.66	1.11 ± 0.29	2.61 ± 0.83	0.790	0.360	0.075

[a] All values are mean ± SEM. wt% = (μg of each fatty acid/μg total fatty acids of platelet phospholipids) × 100%. Total platelet phospholipid omega 6 polyunsaturated fatty acids (n-6 PUFA) = (LA + GLA + DGLA + AA); LA, linoleic acid; GLA, gamma linolenic acid; DGLA, dihomo-gamma linolenic acid; AA, arachidonic acid. Total platelet phospholipid omega 3 polyunsaturated fatty acids (n-3 PUFA) = (ALA + EPA + DHA); ALA, alpha linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SHHF, spontaneous hypertension heart failure; ANOVA, analysis of variance.
[b] Obese male is significantly different from obese female, $P < 0.05$.
[c] Obese is significantly different from lean of the same sex, $P < 0.05$.

relationship may have physiologic significance in that lag time may shorten under pro-aggregatory metabolic conditions. Platelet aggregation (impedance) showed significant positive correlation with AA (Fig. 2A), total n-6 PUFA (Fig. 2B), and serum cholesterol ($R = 0.56$, $P = 0.025$), whereas lag time showed a significant inverse correlation with both AA and n-6 PUFA (Fig. 3A and B). There was no relationship between total n-3 PUFA and platelet aggregation or lag time. Neither impedance nor lag time were significantly correlated with either platelet count or MPV.

Discussion

In the SHHF rat model, there is an effect of male sex and obesity on platelet PUFA, resulting in increased platelet AA and total n-6 PUFA in OM rats. This was accompanied by marked hypercholesterolemia only in the OM. Obesity and diabetes have been associated with similar increases in platelet AA in humans (43–46). Obese male SHHF rats resemble type 2 diabetics and have markedly increased circulating insulin and glucose levels (29), which might influence platelet AA and n-6 PUFA composition. The increased platelet AA level in the diabetic condition may be explained by stimulated AA incorporation into platelets (47–49). Takahashi *et al.* (48) demonstrated that the AA incorporation into platelet phospholipids was significantly higher in type 2 diabetic patients than in age-matched control subjects. Tretjakovs *et al.* (49) also observed similar results, where the incorporation of AA into phospholipids was greater in coronary artery disease patients with type 2 diabetes compared to nondiabetics.

Similar high levels of n-6 PUFA and AA in platelets were not observed when female rats were obese, and the OFs had platelet PUFA and AA content comparable to LFs. This suggests that female sex hormones may protect against some of the metabolic consequences of obesity or that male sex hormones may promote increased AA incorporation into platelet membranes. Studies have suggested that the female hormone, 17-beta-estradiol, inhibits AA and cholesterol synthesis (34, 50). In contrast to this study with SHHF rats, we found in a previous study that obese women with elevated circulating insulin levels had increased platelet AA compared to lean, normoinsulinemic women (43). Their insulin level positively correlated with the increased AA

Table 3. Hematology and Platelet Aggregation in SHHF Rats[a]

	Obese SHHF		Lean SHHF		Two-factor ANOVA *P* value		
	Male	Female	Male	Female	Sex	Obesity	Sex × obesity
Hematocrit (%)	40.6 ± 1.9	40.3 ± 0.6	42.3 ± 0.9	37.2 ± 1.1	0.079	0.546	0.130
Platelet count ($\times 10^9$/l)	800 ± 86	721 ± 46[c]	688 ± 49	569 ± 93	0.015	0.002	0.585
Mean platelet volume (fL)	5.3 ± 0.2	5.4 ± 0.1[c]	5.2 ± 0.1[b]	4.6 ± 0.1	0.265	0.014	0.061
Platelet aggregation (Impedance: ohms)	9.9 ± 1.0	6.9 ± 1.4	8.3 ± 0.7	6.4 ± 1.0	0.043	0.365	0.584
Lag time (min)	0.83 ± 0.14	1.27 ± 0.24	0.98 ± 0.09	1.10 ± 0.06	0.082	0.948	0.305

[a] All values are mean ± SEM. SHHF, spontaneous hypertension heart failure; ANOVA, analysis of variance.
[b] Lean male is significantly different from lean female, $P < 0.05$.
[c] Obese is significantly different from lean of the same sex, $P < 0.05$.

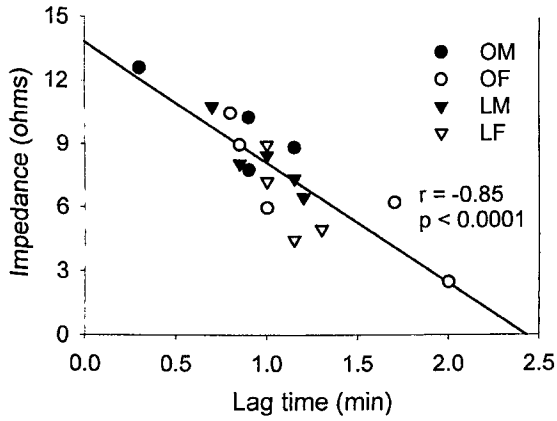

Figure 1. Using whole blood aggregometry, impedance was significantly inversely correlated with lag time to initiation of aggregation in SHHF rats ($r = -0.85$, $P < 0.0001$). Obese male (OM, filled circle), obese female (OF, open circle), lean male (LM, filled triangle), and lean female (LF, open triangle).

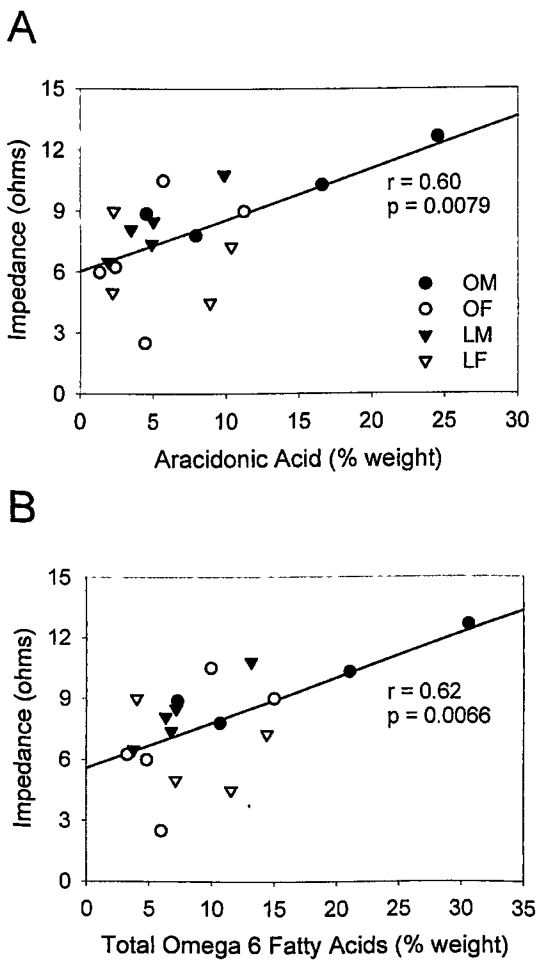

Figure 2. Impedance by whole blood aggregometry was significantly correlated with platelet phospholipid (A) arachidonic acid content ($r = 0.60$, $P = 0.0079$) and (B) total n-6 polyunsaturated fatty acid content ($r = 0.62$, $P = 0.0066$) in SHHF rats. Obese male (OM, filled circle), obese female (OF, open circle), lean male (LM, filled triangle), and lean female (LF, open triangle) SHHF rats.

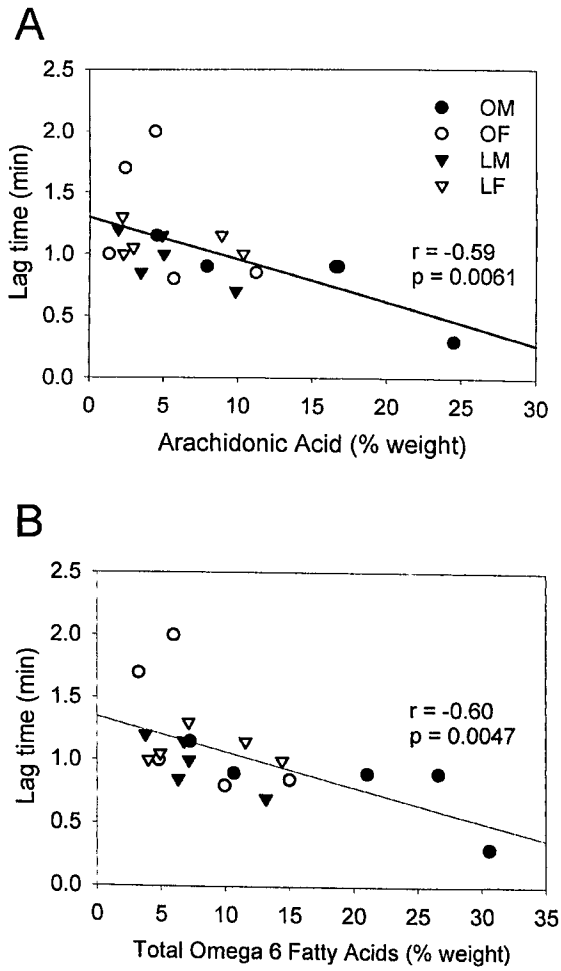

Figure 3. Lag time to initiation of platelet aggregation was significantly inversely correlated with platelet phospholipid (A) arachidonic acid content ($r = -0.59$, $P = 0.0061$) and (B) total n-6 polyunsaturated fatty acid content ($r = -0.60$, $P = 0.0047$) in SHHF rats. Obese male (OM, filled circle), obese female (OF, open circle), lean male (LM, filled triangle), and lean female (LF, open triangle) SHHF rats.

level in their platelets, and insulin, but not estrogen, correlated with indexes of desaturase function.

During platelet aggregation, AA is liberated from platelet membrane phospholipids and then subsequently oxidized into TXA_2, a potent aggregating agent (6, 8, 21). Increased AA content or AA metabolism in platelets is highly correlated with platelet hyperfunction(49, 52–55). Similarly, there were significant positive correlations between *in vitro* platelet aggregation in response to collagen and both platelet AA and total n-6 content in 6-month-old SHHF rats, whereas lag time was inversely correlated to both AA and total n-6 PUFA. Although it was not a significant difference, there was a trend for aggregation to be enhanced and lag time to be shorter in the OMs, which had the highest platelet AA levels. This suggests that greater platelet AA content in the OMs is pro-aggregatory. The augmented level of pro-aggregatory metabolite of AA, such as TXA_2, released from activated platelets and its interaction

with vascular system plays an important role in pathogenesis of cardiovascular diseases (7, 12, 56, 57). The earlier onset of cardiovascular abnormalities in OM rats as compared to OF, LM, and LF rats may partially be explained by these phenomena of altered platelet AA contents and aggregation.

Though increased n-6 PUFA (58) or AA incorporation (49, 55) into platelet phospholipids is associated with platelet hyperfunction, many studies with humans or animals reported that higher n-3 PUFA consumption either reduces platelet aggregation, platelet AA level, or TXB_2 production—the stable metabolite of TXA_2 (20, 59–62). However, a connection between platelet n-3 fatty acid level and platelet activity has not been demonstrated in some studies (45, 63–66). In our study, we also did not observe a correlation between EPA or total n-3 PUFA levels and platelet aggregation.

Obesity was also associated with marked elevated circulating triglycerides in both male and female SHHF. It has been suggested that hypertriglyceridemia might accelerate and increase platelet aggregation in response to collagen stimulation (18, 67). In contrast, Aoki *et al.* (68) demonstrated that hypercholesterolemia, but not hypertriglyceridemia, was associated with increased platelet-dependent thrombin generation. In SHHF rats, in spite of hypertriglyceridemia, OFs did not show increased AA, total n-6 PUFA, and platelet aggregation, unlike OMs. Again, this suggests that female sex hormones may protect against the effects of hypertriglyceridemia or that factors other than increased serum triglycerides, such as hypercholesterolemia, may have contributed to platelet function and PUFA profile differences in OM SHHF rats.

The role of leptin resistance and hyperleptinemia in promoting platelet activation in obesity remains controversial (69–73). Leptin levels are elevated in most obese individuals as well as in obese SHHF rats (24, 26, 72). A few studies demonstrated that leptin stimulated aggregation in a dose-dependent manner in murine and human platelets (69–72). In contrast, Ozata *et al.* (73) reported that higher concentrations of leptin do not increase platelet aggregation in humans. Obese SHHF rats in both genders have elevated serum leptin levels (24; and unpublished data). However, only OMs tended to increase platelet aggregation. Therefore, if leptin is a factor stimulating platelet aggregation, its effect may be modified by sex.

Female sex was associated with lower platelet numbers and smaller circulating platelet size, compatible with overall decreased platelet mass in the LFs. This may be due to the suppressive effects of estrogen on hematopoietic precursors (74–76), though the role of estrogen on platelet production is uncertain. Obesity abolished the sex differences observed in platelet count and MPV. Though not measured in this study, it is likely that OF SHHF rats have abnormal production of sex hormones, as evidenced by irregular cycling and impaired fertility (31). Lean females also showed less cardiac hypertrophy compared to LMs. This appeared to be independent of the degree of hypertension, as there were no differences in blood pressure among groups. Obesity abolished the male/female effect on heart size, and OFs had similar heart to brain weight ratios as the OMs.

In conclusion, we have observed an effect of male sex and obesity resulting in greater platelet AA and n-6 PUFA levels in the SHHF model of heart failure prone rat. Alterations in AA and n-6 PUFA have functional significance in promoting platelet activation and aggregation. Female sex appeared to abrogate some of the deleterious effects of obesity on n-6 PUFA and AA metabolism, and may have consequences on progression of cardiovascular disease in this rat model.

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