Predictive Adaptive Responses to Maternal High-Fat Diet Prevent Endothelial Dysfunction but Not Hypertension in Adult Rat Offspring

Imran Khan, PhD; Vasia Dekou, MSc; Mark Hanson, DPhil; Lucilla Poston, PhD; Paul Taylor, PhD

Background—Population-based studies suggest that fetal adaptive responses to maternal dietary imbalance confer survival benefit when the postnatal diet remains suboptimal but increase susceptibility to cardiovascular disease when postnatal nutrition is improved. We have investigated “predictive adaptive” responses in a rodent model in which adult offspring of fat-fed dams develop characteristics of the metabolic syndrome.

Methods and Results—Sprague-Dawley rats were fed a fat-rich diet or normal chow throughout pregnancy and weaning. Vascular endothelial function and blood pressure were determined in 180-day-old offspring of fat-fed dams raised on standard chow (FC) or on the fat-rich diet (FF) and in offspring of chow-fed dams raised on chow (CON). Small mesenteric artery endothelium-dependent dilation to acetylcholine was impaired in male and female FC (by ANOVA, $P<0.001$ versus CON) but similar to CON in FF ($P=NS$). Blood glucose was lower in FF versus FC. Heart rate was reduced in male FC versus CON ($P<0.05$) but not in FF. Plasma triglyceride concentrations were reduced in male FF compared with CON ($P<0.05$). Blood pressure was raised in female FC (systolic, $132.5+3.0$ mm Hg versus CON, $119.0+3.8$ mm Hg, $P<0.05$; diastolic, $91.2+1.7$ mm Hg versus CON, $81.1+1.4$ mm Hg, $P<0.05$) and in female FF (systolic, $132.5+4.2$ mm Hg versus CON, $P<0.05$; diastolic, $91.0+1.9$ mm Hg versus CON, $P<0.05$). Blood pressure was similar to CON in male FC and FF.

Conclusions—Predictive adaptive responses prevent endothelial dysfunction and reduced heart rate in offspring of fat-fed dams if offspring are raised on the same diet but do not prevent development of raised blood pressure. (Circulation. 2004;110:1097-1102.)

Key Words: diet ■ endothelium ■ blood pressure ■ telemetry ■ pregnancy

An adverse prenatal nutritional environment is increasingly recognized to enhance susceptibility to adulthood cardiovascular disease. Robust evidence in support of the concept of developmental “programming” lies in the association of low birth weight with adulthood hypertension, type 2 diabetes, and other features of the metabolic syndrome. This relationship is most pronounced among offspring showing a rapid growth trajectory in early childhood and has led to the “thrifty phenotype” hypothesis, which proposes that the growth-restricted fetus is programmed in utero for a life of nutritional hardship. The hypothesis suggests that prenatally acquired predisposition to insulin resistance will confer survival benefit if postnatal nutrition is poor but that it will predispose the individual to development of the metabolic syndrome should the postnatal diet be plentiful. Developmental “predictive adaptive” responses to an adverse in utero environment can therefore prove to be maladaptive in adulthood if dietary conditions change. Predictive adaptive responses are also observed in other adverse environmental conditions; eg, it is reported that sweat gland density is determined in early postnatal life by environmental temperature, an adaptation that may determine the degree of tolerance to extremes of climate later in life.

Developmental programming of cardiovascular and metabolic risk factors is readily observable in animal models of nutrient restriction. Rodents exposed in early development to maternal protein deprivation or maternal global dietary restriction demonstrate insulin resistance and hypertension in adulthood when maintained on a balanced diet. Developmental programming of adulthood cardiovascular and metabolic disorders in animals is, however, not limited to dietary restriction, because we and others have shown that exposure to a maternal diet rich in saturated fat or cholesterol also results in features of the metabolic syndrome, vascular dysfunction, and vascular atherogenic lesions in adult animals raised on standard chow.

Despite the suggestion that fetal predictive adaptive responses to a maternal imbalanced diet favor adulthood...
survival on a similar diet, the hypothesis has not been adequately tested in animal models of developmental programming. We have therefore determined whether the cardiovascular defects observed in normally fed adult rats previously exposed through gestation and weaning to a maternal fat-rich diet are still evident if the offspring are maintained on their habituated, and therefore predicted, fat-rich diet or whether adaptive mechanisms program cardiovascular protection against the adulthood dietary insult. Parameters of cardiovascular and metabolic function were investigated in offspring of dams fed a diet rich in animal lard during pregnancy and lactation. One group of offspring were maintained on the same diet throughout development and until 180 days of age, whereas another was maintained on a balanced diet. Comparison was made with control animals exposed to a balanced diet during pregnancy and lactation.

Methods
Female Sprague-Dawley rats (Harlan, UK) 100 to 120 days old were fed, for 10 days before mating and throughout pregnancy and lactation, either a control breeding diet (n = 10) of standard laboratory chow (5.3% fat, corn oil, 21.2% protein, 57.4% carbohydrate, 4.6% fiber; Rat and Mouse Diet No. 3 [RM3], Special Diet Services) or the same diet supplemented wt/wt with animal lard (n = 10) and with added vitamins, minerals, and protein to achieve a similar composition of these components to the control diet (final analysis: 10% or the same diet supplemented wt/wt with animal lard (n = 10) and with added vitamins, minerals, and protein to achieve a similar composition of these components to the control diet (final analysis: 25.7% fat, 19.5% protein, 41.3% carbohydrate, 3.5% fiber; estimated fats: palmitic acid 4.50%, stearic acid 1.99%, palmitoleic acid 0.12%, oleic acid 6.86%, linoleic acid 2.58%, α-linolenic acid 0.25%, arachidonic acid 0.19%; Special Diet Services). Composition was confirmed by independent analysis (Eclipse Scientific Group). Maternal weight and food intake were measured daily. Two days postpartum, litters were reduced to 8 pups and, when possible, to equal numbers of males and females. After weaning (21 days), offspring of fat-fed dams were fed ad libitum on standard chow (FC, n = 10) or on the lard-supplemented diet (FF, n = 5). Offspring of control-fed animals (CON, n = 10) were maintained on standard chow. Food intake and animal weights were recorded from 1 week of age (to avoid maternal rejection of the pups) until animals were fully grown. All animals were housed with controlled temperature and humidity on a 12-hour light/dark cycle. All animal care guidelines and animal procedures followed were licensed under the UK Home Office Animal (Scientific Procedures) Act 1986.

Assessment of Offspring Blood Pressure, Heart Rate, and Activity by Radiotelemetry
Blood pressure, heart rate, and activity were assessed by radiotelemetry (Dataquest IV, Data Sciences International) in 180-day-old FC, FF, and CON as described previously. One male and 1 female from each litter were studied. Variables were recorded every 10-second intervals for 5 minutes. Twelve-hour day and night mean values were computed. Animals were then fasted overnight and killed by CO₂ inhalation. Abdominal fat lobes were excised and weighed. Blood samples for total cholesterol, triglycerides, HDL, glucose, and insulin were obtained by cardiac puncture, and plasma was stored at −70°C.

Assessment of Mesenteric Resistance
Artery Function
Vascular function was assessed in 180-day-old littermates of the FF, FC, and CON rats used for telemetric recording. Rats were killed by CO₂ inhalation and cervical dislocation. Third-order branches of the mesenteric arcade were dissected and mounted on a small-vessel myograph as described previously. Arteries were submaximally constricted with norepinephrine (80% of maximal concentration), and concentration responses to acetylcholine (10⁻⁸ to 10⁻⁵ mol/L) and aqueous nitric oxide (10⁻⁷ to 10⁻⁵ mol/L) were determined as described previously. Acetylcholine was obtained from Sigma, norepinephrine from Abbott Laboratories, and NO gas from BDH.

Plasma Analyses
Plasma glucose was measured by a routine laboratory enzymatic UV test (HK/G6P-DH method; Cobas Farab Centrifugal analyzer), and insulin by ELISA (DRG Instruments GmbH). Plasma triglyceride and total cholesterol concentrations were measured by enzymatic colorimetric assays (UNIMATE 5 TRIG and UNIMATE 5 CHOL, Roche/BCL).

Statistical Analysis
Radiotelemetry
Multiple regression with generalized estimating equations was used to estimate differences between groups and to test for interactions between groups and sex of offspring. Statistical significance was assumed at a value of P < 0.05. Weekly blood pressures, heart rates, and activity were calculated by averaging the mean values obtained for each night and day period.

Vascular Function and Plasma Analyses
Relaxation responses were calculated as a percentage of initial preconstriction to norepinephrine, and statistical comparisons of the concentration-response curves were made by repeated-measures ANOVA. EC₅₀s and maximal responses were compared by Student’s t test with Bonferroni correction for multiple comparisons. All values are given as mean ± SEM. Statistical significance was assumed at a value of P < 0.05.

Other Parameters
Food intake, body weight, and total fat depot weights were measured and compared between groups by repeated-measures ANOVA.

Results
Radiotelemetric Monitoring of Offspring Blood Pressure, Heart Rate, and Activity
Female
Systolic blood pressure was higher in female FC than CON (FC, 132.5 ± 3.0 mm Hg, n = 7, versus CON, 119.0 ± 3.8 mm Hg, n = 7, P < 0.05; Figure 1A). Raising the pregnantly fat-exposed animals on a fat-rich diet did not prevent or exacerbate this increase in blood pressure because systolic pressure was similarly raised in female FF (FF, 132.5 ± 2.2 mm Hg, n = 5 versus FC, P = NS; versus CON, P < 0.05; Figure 1A). Diastolic blood pressure was also higher than CON in female FC (FC, 91.2 ± 1.7 mm Hg versus CON, 81.1 ± 1.4 mm Hg; P < 0.025) and female FF (91.0 ± 1.9 mm Hg versus CON; P < 0.05; Figure 1A). Locomotor activity (data not shown) and heart rate (Figure 2A) were similar in female FF, FC, and CON.

Male
There were no significant differences in blood pressure (Figure 1B) or locomotor activity (data not shown) between male FF, FC, and CON offspring. Heart rate was lower than CON in male FC (FC, 355.4 ± 9.5 bpm, n = 6 versus CON, 398.3 ± 5.2 bpm, n = 6; P < 0.05; Figure 2B), but this difference was not apparent in fat-fed male offspring (FF versus CON, P = NS), which demonstrated a higher heart rate than male FC (FF, 390.2 ± 5.8 bpm, n = 5; versus FC, P < 0.05; FF versus CON, P = NS).
Endothelium-Dependent Relaxation
Relaxation to acetylcholine (Figure 3) was impaired in third-order mesenteric arteries of 180-day-old male FC compared with CON \( (P<0.001 \text{ versus CON by ANOVA}; \text{maximum relaxation, } 67.92\pm2.89\% \text{ versus CON, } 92.08\pm2.19\%; \text{ } P<0.01) \) and also in female FC \( (P<0.001 \text{ versus CON by ANOVA}; \text{maximum relaxation, } 75.24\pm7.56\% \text{ versus CON, } 93.74\pm2.08\%; \text{ } P<0.05) \). This defect was not apparent in the male or female fat-fed offspring of fat-fed dams (FF), with both males and females demonstrating significantly greater relaxation than FC \( (\text{male FF versus FC, } P<0.001 \text{ by ANOVA}; \text{maximum relaxation, } 88.13\pm0.23\% \text{ versus FC, } P<0.01; \text{ female FF, } P<0.001 \text{ versus FC by ANOVA and maximum relaxation, } 91.64\pm3.44\% \text{ versus FC, } P<0.01). \) Responses in male and female FF were not significantly different from CON groups \( (P=\text{NS by ANOVA}) \) (Figure 3, A and B).

Endothelium-Independent Relaxation

Males and Females
Endothelium-independent relaxation as assessed by responses to aqueous nitric oxide was not different between groups \( (P=\text{NS by ANOVA}; \text{maximum relaxation: males, FC, } 95.20\pm2.34\%, \text{ } n=8; \text{ FF, } 95.40\pm1.96\%, \text{ } n=5; \text{ CON, } 95.24\pm1.97\%, \text{ } n=8; \text{ females, FC, } 96.22\pm2.81\%, \text{ } n=9; \text{ FF, } 96.54\pm1.23\%, \text{ } n=5; \text{ CON, } 98.36\pm0.82\%, \text{ } n=8). \)

Plasma Parameters
Results are shown in the Table. Male FF demonstrated reduced plasma triglyceride concentration compared with CON. The fasting plasma glucose concentration was raised in male FC versus CON \( (P<0.05) \) but not in FF versus CON. The fasting plasma insulin concentration was increased in male FF versus CON \( (P<0.05) \). All other parameters were not different between groups.

Figure 1. Systolic (SBP) and diastolic (DBP) blood pressure recorded for 1 week (night, N, and day, D) in 180-day-old female (A) and male (B) offspring. Offspring of control dams (CON, open circle, \( n=6 \)), offspring of fat-fed dams maintained on normal chow (FC, solid circle, \( n=6 \)), and offspring of fat-fed dams maintained on fat-rich diet (FF, shaded circle, \( n=5 \)). Values are expressed as mm Hg, mean±SEM. Black asterisk, \( P<0.05 \) CON versus FC, and gray asterisk, \( P<0.05 \) FF versus CON by generalized estimating equation.

Figure 2. Heart rate in 180-day-old female (A) and male (B) offspring. Offspring of control dams (CON, open circle, \( n=7 \)), offspring of fat-fed dams maintained on normal chow (FC, solid circle, \( n=7 \)), offspring of fat-fed dams maintained on a fat-rich diet (FF, shaded circle, \( n=5 \)). Values are expressed as bpm, mean±SEM. Black asterisk, \( P<0.05 \) CON versus FC, and gray asterisk, \( P<0.05 \) FF versus FC by generalized estimating equation.

Figure 1. Systolic (SBP) and diastolic (DBP) blood pressure recorded for 1 week (night, N, and day, D) in 180-day-old female (A) and male (B) offspring. Offspring of control dams (CON, open circle, \( n=6 \)), offspring of fat-fed dams maintained on normal chow (FC, solid circle, \( n=6 \)), and offspring of fat-fed dams maintained on fat-rich diet (FF, shaded circle, \( n=5 \)). Values are expressed as mm Hg, mean±SEM. Black asterisk, \( P<0.05 \) CON versus FC, and gray asterisk, \( P<0.05 \) FF versus CON by generalized estimating equation.

Figure 2. Heart rate in 180-day-old female (A) and male (B) offspring. Offspring of control dams (CON, open circle, \( n=7 \)), offspring of fat-fed dams maintained on normal chow (FC, solid circle, \( n=7 \)), offspring of fat-fed dams maintained on a fat-rich diet (FF, shaded circle, \( n=5 \)). Values are expressed as bpm, mean±SEM. Black asterisk, \( P<0.05 \) CON versus FC, and gray asterisk, \( P<0.05 \) FF versus FC by generalized estimating equation.
Female, n 7 6 5
Maintained on Fat-Rich Diet (FF) at 180 Days of Age

Body Weight, Adiposity, and Food Intake

Body weight in the male lard-fed offspring of the lard-fed dams was significantly increased relative to controls at 180 days. It is known, for example, that if rats are exposed to a fat-rich diet during pregnancy and suckling to a diet rich in lard could be protective of vascular defects described previously in adult rats exposed to maternal hypercholesterolemia, gives rise to an offspring phenotype predisposed to development of adulthood cardiovascular disease.11–15 The present study investigated whether any or all of the cardiovascular defects described previously in adult rats exposed during pregnancy and suckling to a diet rich in lard could be prevented if the rats were fed the same diet in adulthood, ie, to investigate the role of predictive adaptive responses. Remarkably, the defect in endothelial function in male and female offspring of rats exposed prenatally and during suckling to the fat-rich diet and maintained from weaning on standard chow was absent when the offspring were fed the fat-rich diet. Similarly, the fall in heart rate in male offspring maintained on standard chow was not apparent in the offspring fed the fat-rich diet. In contrast, the increase in blood pressure, which was confined to the female offspring, remained elevated when female offspring were fed the fat-rich diet. Furthermore, early exposure to the fat diet did not prevent an increase in adiposity in either sex maintained on the same diet. These data therefore support only in part the theory that the fetus or neonate is capable of mounting a predictive adaptive response to an anticipated postnatal diet. The endothelial dysfunction and the sex-linked hypertension in offspring maintained on standard chow confirm our earlier report.14

Few investigators have previously studied predictive adaptive responses in the different animal models of developmental programming. Despite the frequent use of the maternal protein restriction and global dietary restriction models for investigation of the thrifty phenotype hypothesis, in which adult offspring maintained on standard chow develop hypertension and insulin resistance, it is not established whether the offspring are protected from these disorders if raised on the maternal diet. It is known, for example, that if rats are

Discussion

A growing body of literature from human studies and animal models suggests that exposure to maternal diets rich in fat during pregnancy and suckling, or to maternal hypercholesterolemia, gives rise to an offspring phenotype predisposed to development of adulthood cardiovascular disease. This phenomenon is known as the thrifty phenotype hypothesis (TPH). TPH posits that exposure to an adverse environment during fetal and postnatal development leads to a metabolic phenotype that is predisposed to the development of cardiovascular disease in adulthood. The TPH hypothesis has been supported by findings in animal models where offspring of mothers that were exposed to a fat-rich diet during pregnancy and suckling developed increased body weight, adiposity, and cardiovascular risk factors in adulthood compared to control offspring. These changes were often seen in both male and female offspring, although the magnitude and timing of the responses could vary by sex.

In the present study, investigators investigated whether exposure to a fat-rich diet during pregnancy and suckling, or to maternal hypercholesterolemia, would result in an offspring phenotype predisposed to development of adulthood cardiovascular disease. They compared the effects of maternal exposure to a fat-rich diet on body weight, adiposity, and cardiovascular function in male and female offspring. The results showed that offspring of fat-fed dams fed fat-rich diet (FF) at 180 days of age had significantly increased body weight and adiposity compared to controls. This was true for both male and female offspring, with male offspring showing a greater increase in body weight compared to female offspring. Additionally, endothelial dysfunction, as measured by a decreased response to acetylcholine, was observed in the offspring of fat-fed dams compared to control offspring.

The study also measured other cardiovascular risk factors, including plasma lipids and glucose levels. Offspring of fat-fed dams had elevated triglyceride, cholesterol, and glucose levels compared to control offspring. These findings are consistent with previous studies that have shown a relationship between maternal diet and offspring cardiovascular risk factors.

The authors concluded that exposure to a fat-rich diet during pregnancy and suckling, or to maternal hypercholesterolemia, resulted in an offspring phenotype predisposed to development of adulthood cardiovascular disease. This finding supports the TPH hypothesis and highlights the importance of maternal diet during pregnancy and suckling on the development of cardiovascular risk factors in offspring. However, the study also noted that the effects of maternal diet on offspring cardiovascular health may be sex-specific, with male offspring showing a greater response than female offspring.

In conclusion, the present study provides evidence for the TPH hypothesis and highlights the importance of maternal diet during pregnancy and suckling on the development of cardiovascular risk factors in offspring. Further research is needed to understand the underlying mechanisms and to develop interventions to prevent the development of cardiovascular disease in adulthood.
exposed to a protein-restricted diet prenatally and until adulthood (71 days) and then fed a balanced diet, insulin resistance and hypertension ensue, but it is not recorded whether these disorders are prevented by continued feeding of the restricted diet to which the fetus is habituated. Indeed, there is some evidence contrary to the thrifty phenotype hypothesis, because mice exposed to protein restriction during the suckling period alone develop sustained protection against obesity when fed a “cafeteria” diet. Rats that are protein restricted during weaning also demonstrate a greater life span than controls when maintained on a normal diet.

An investigation in which pig sows were fed an atherogenic diet in pregnancy has more relevance to the present study. Pregnant sows were fed a diet supplemented with beef tallow (31.7% fat versus 7.4% for standard diet). Control piglets and piglets prenatally exposed to the fat diet were maintained on either a standard diet or the “atherogenic” diet and studied when prepubertal (5 months). All offspring fed the atherogenic diet demonstrated higher serum cholesterol concentrations than controls. However, piglets from sows fed the standard diet developed early coronary atherosclerosis when maintained on the atherogenic diet, whereas those prenatally challenged with the fatty diet and maintained on the same diet had no evidence of aortic lesions despite being hypercholesterolemic. That study thus clearly suggested that predictive adaptive responses to the maternal diet protect against a subsequent dietary fat insult. Rats are recognized for resistance to the development of atherosclerosis, and we did not investigate aortic fat deposition in the present study.

However, because there is an established association between endothelial dysfunction and atherogenesis and because endothelial function was normal only in the rats challenged with the fat-rich diet throughout life, mechanistic similarities may exist between the rat and pig models.

The adverse consequences of fat-feeding on endothelial function are well documented, and we have shown previously a significant decrease in relaxation to acetylcholine in small mesenteric arteries of virgin female rats fed a diet supplemented with animal fat. Because the splanchnic circulation contributes to peripheral vascular resistance, these arteries, which are amenable to the technique of myography, are frequently used in investigations of the systemic vasculature.

From the present results and our previous observations, it can be deduced that exposure in utero and in suckling to dietary animal fat confers protection against endothelial dysfunction associated with dietary fat exposure in adulthood. The mechanism underlying endothelial dysfunction subsequent to developmental exposure to the fat-rich diet in chow-fed offspring is not known, although in older animals (360 days) studied previously, the defect was accompanied by reduced HDL concentrations in both sexes and elevation of triglyceride and glucose concentrations in females. Two endothelial “protective” trends were apparent in the present study in the offspring of the fat-fed dams maintained on the fat-rich diet; both the plasma glucose and triglyceride concentrations were reduced in males, which may be a reflection of improved insulin sensitivity. Accurate evaluation of insulin sensitivity and determination of relevant endothelial gene expression may provide further insight into the preventive
mechanism, as would the measurement of the vascular fatty acid profile, which we have previously shown to be abnormal in prenatally fat-exposed offspring maintained on a normal diet.24

The significant fall in heart rate compared with controls observed in male offspring of the fat-fed dams maintained on standard chow may in part explain the contrasting data in the females, which demonstrated raised blood pressure but normal heart rate. The normality of the heart rate in the dietary-challenged male offspring that were maintained on the fat-rich diet provides further evidence for predictive adaptive responses in early life.

In contrast to endothelial function and heart rate abnormalities, the hypertension induced in female offspring by previous exposure to maternal dietary fats persisted with continued fat-feeding. This may imply an irreversible, adverse effect of the maternal diet on pathways of blood pressure control. Others have shown that male Wistar rats fed lard develop raised blood pressure,25 and it is possible that blood pressure would have been raised further if the animals had not experienced developmental exposure to the diet. Nonetheless, the females were not adapted in early life to remain normotensive when maintained on the same diet as fed to their dams.

Ongoing studies of this model include investigation of the pathways that lead to developmental programming of the adulthood disorders. Maternal hyperinsulinemia and alteration to the maternal and fetal hypothalamic-pituitary-adrenocortical axis may provide the initial stimulus,26 and preliminary studies in our model have recently proposed a pivotal role for mitochondrial dysfunction.

The importance of this study is 3-fold. First, the data suggest that early predictive adaptive responses to excesses of maternal animal fat intake in utero and during suckling only partially protect the offspring against programmed cardiovascular risk when exposed to the same fat-rich diet in later life. Second, they provide some of the first evidence for predictive adaptive responses in the fetus/neonate to a maternal fat-rich diet in an animal model. This occurs without the influence of the varied genetic background that confounds studies in humans. Third, it emphasizes the need for investigations in developmental programming to include groups of experimental animals maintained on the same diet as that of the dam and, in addition, to examine the range of tolerance to variations in the adult diet. Studies of this design are likely to be more valuable to our understanding of the range of tolerance to variations in the adult diet. Studies of

Acknowledgments
This study was supported by the British Heart Foundation. Professor Poston is supported by Tommy’s the Baby Charity.

References
Predictive Adaptive Responses to Maternal High-Fat Diet Prevent Endothelial Dysfunction but Not Hypertension in Adult Rat Offspring

Imran Khan, Vasia Dekou, Mark Hanson, Lucilla Poston and Paul Taylor

_Circulation._ 2004;110:1097-1102; originally published online August 23, 2004;
doi: 10.1161/01.CIR.0000139843.05436.A0
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/110/9/1097

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/