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Basic nutritional investigation

Effects of maternal high-fat diet and statin treatment on bone marrow endothelial progenitor cells and cardiovascular risk factors in female mice offspring fed a similar diet

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ABSTRACT

Objectives: The aim of this study was to prove that one possible statin-related protective mechanism in dams and offspring fed a high-fat diet (HFD) is the reduction in cardiovascular risk and impairment of the vasculogenic element of endothelial regeneration.
Methods: To explore this, virgin C57 BL/6 mice (n = 8/group) were fed an HFD (fat: 45% kcal) or standard chow (C; fat: 21% kcal) from weaning and throughout their pregnancy and lactation. Half of the HFD group also was given the 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor pravastatin (S) through their drinking water (5 mg/kg body weight per day) to create HF+S dam group (n = 8/group). Offspring from each group were fed HFD or C diet from weaning to adulthood, generating respective dam/offspring dietary groups (C/C, HF/HF, HF+S/HF; n = 8/group). Body weight, blood pressure, and serum lipid profile were measured in female offspring at age 24 wk, and bone marrow endothelial progenitor cells (EPCs) were cultured.
Results: The results indicated that in the female offspring, the statin-fed (HF+S/HF) cohort had lower total and low-density lipoprotein cholesterol concentrations, were less obese and hypertensive, and had reduced C-reactive proteins (CRPs) compared with the HF/HF phenotype. The results also showed an increased bone marrow EPCs expressing colony numbers ($P < 0.001$) compared with the HF/HF phenotype.
Conclusions: Results from the present study demonstrated that statin administration in early life to dams fed on a HFD had a significant effect on their female offspring in terms of reduction in cardiovascular risk factors. Additionally, statin administration to female offspring on an HFD during early life was associated with reduction in circulating CRPs and an increased bone marrow EPC numbers and colony-forming characteristics.

Introduction

Endothelial dysfunction is a common phenomenon that occurs in the metabolic syndrome [1,2]. Evidence suggests that diabetes and hyperlipidemia lead to reduced circulation of blood and bone marrow-derived mononuclear cells, that is endothelial progenitor cells (EPCs), thus resulting in endothelial cell dysfunction [3–6]. Emerging reports also suggest endothelial dysfunction (i.e., reduced EPC) as a common phenotype in a number of rodent models of both maternal high-fat (HF) and total nutrient restriction in the context of developmental origins of health and disease (DOHaD) models [7,8].

In response to marked morphologic changes in the surrounding mature endothelial cells, EPCs play a critical role in maintaining endothelial function in mature blood vessels

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by contributing to reendothelialization and neovascularization [9,10]. It is therefore conceivable that the mobilization and differentiation of EPCs are important in this process of adult neovascularization [11–13] and any impairment of this vasculogenic element of endothelial regeneration may account for the progression of endothelial dysfunction [13].

Accumulating evidence also suggests that the number and migratory activity of circulating EPCs inversely correlate with circulating C-reactive protein (CRP) levels [4,14]. The combination of reduced circulating EPCs and increased circulating CRP is known to be associated with metabolic syndrome (MetS) and high low-density lipoprotein cholesterol (LDL-C) [15,16]. Reports have advocated that CRP downregulates endothelial nitric oxide synthase (eNOS) in a synchronous fashion and destabilizes eNOS mRNA transcription, decreases both basal and stimulated nitric oxide (NO) release [17], upregulates nuclear factor-κB, a key nuclear factor that facilitates the transcription of numerous proatherosclerotic genes [18], and mediates adhesion molecules and LDL uptake [19,20].

These interrelations among LDL-C, CRP, and bone marrow EPCs, suggest that CRP-related alteration in progenitor cell number and function in offspring may be induced by maternal HF consumption. Also, it would be interesting to study, through net reduction effect on maternal dyslipidemia load, whether statin therapy in dams exerts any advantageous effect on bone marrow EPC in their offspring fed a similar high-fat diet (HFD). Several experimental models and in vivo studies on patients with ischemic disease support these hypotheses [21,22]. Although the precise mechanisms remain unclear, it is known that statin improves endothelial function by activating protein kinase Akt [21], mobilizing EPC [23], reducing senescence, and increasing proliferation of EPC [24].

A major area of DOHaD research provides a novel explanation on disturbances in the maternal metabolism resulting from altered nutrient supply in the mother, a trait that is transmitted to the fetus in the form of structural and functional adaptations during fetal development and throughout life [25–28]. Consequently, we developed a mice model [29,30] for studying the effect of altered maternal nutrients supply in the mother transmitted to the fetus during development. In these studies [29,30], we reported that statin administration during the second half of pregnancy and lactation in dams consuming an HFD reduces metabolic risk factors not only in dams but also in their offspring [31]. These favorable effects were more prominent in the offspring of HF dams who had statin treatment at the time they were weaned, during pregnancy and lactation [31]. However, it is not clear what the possible underlying mechanism is. We hypothesize that one such possible protective mechanism in dams and offspring fed an HFD is the reduction in impairment of the vasculogenic element of endothelial regeneration. We believe that statins reduce the levels of circulating CRPs that may be linked with the pathologic mechanisms that regulate bone marrow EPC numbers and their colony-forming properties. The present study was designed to compare the effect of statin with that of other lipid-lowering drugs and to use an experimental design that minimizes the differences in body weight and other parameters induced by an HFD before pregnancy as well as during lactation, as described in more detail in previous publications [29–31]. In brief, using this mice model we had previously explored the effects of long-term consumption of an HFD by dams. The results indicated that HFD in dams predisposes the female offspring to developing a metabolic syndrome-like (MetS-like) phenotype in adult life as indicated by chronic elevation of serum cholesterol and blood pressure. In the follow-up study [30] using the same model, we observed that offspring from HFD dams showed increased adiposity in their femurs in comparison with offspring from dams fed standard chow. In particular, female offspring from dams fed an HFD exhibited altered trabecular structure, indicative of in utero programming. However, the scope covered by all our studies was limited by the availability of funding and other resources, and hence the focus of this study was on MetS-like outcomes. However, the use of this type of model to test the effect of treatment with statins does not find direct relevance to clinical practice in view of the existing controversy concerning the potential high risk of teratogenicity to the unborn fetus [32]. Rather there is no clinical (patients) evidence that statins in pregnancy reduces the risk for anything as they are absolutely contraindicated.

In view of the gap in knowledge, the aim was to study using mice (pregnant dams and their female offspring) fed on a HFD, whether giving statin treatment alters their cardiovascular risk factors and ameliorates impairment of the vasculogenic element of endothelial regeneration.

Materials and methods

Experimental protocol

Study part 1

The aim of this protocol was to resolve the question of whether statins, when administered to pregnant dams on on a HFD, have any effect on the mechanisms of endothelial regeneration. All of the animal procedures were humane and carried out in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986 and the study protocol was approved by the University of Southampton Animal Care and Research Ethics Committee. The experimental protocol for study 1 is as described in Figure 1.

Female C57 BL/6 mice (Charles River Laboratories, United Kingdom) were maintained under a 12-h light/dark cycle at constant temperature (25 ± 2°C) with food and water supplied ad libitum. At 4 wk of age, the females (n = 8/group) were randomly allocated to either a control diet of standard laboratory chow (C; 5.3% fat [corn oil], 21.2% protein, 49.2% carbohydrate; Special Diet Services, United Kingdom) or an experimental HFD (composition of each diet can be found in references [29–31]) supplemented with 18% weight/weight animal lard, with additional vitamins and minerals, protein, and choline to correct for the dilution (final composition in percentage of g [weight/weight]: lard 17.8; casein 26.5; choline chloride 0.3; L-cysteine 0.4; rice starch 28.3; cellulose 6.1; soya oil 4.3; sucrose 10.4; minerals 4.3; and vitamins 1.2; Special Diet Services diet 824053). This HFD has been used in previous studies [33,34]. At 10 wk old, the females were time mated and after confirmation of mating (i.e., presence of vaginal plug), were individually housed. From the second half of the pregnancy and throughout lactation, half of the pregnant females on the HFD were given a water-soluble 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor (pravastatin, Sigma United Kingdom) in their drinking water (HF+S; n = 8/group). Pravastatin was dissolved at a concentration that gave a daily dose of 5 mg/kg, based on the daily water consumption of pregnant and lactating mice predetermined from a previous study [33]. The pregnant dams were allowed to give birth, the pups were weighed, and litter size was standardized to eight pups. After weaning (3 wk postpartum), all female offspring whose dams had been fed diets of C, HF, and HF+S, were themselves randomly allocated to be fed either C or HF. This generated mother and daughter dietary combinations of C/C, C/HF, HF/HF, HF/C; HF+S/HF and HF+S/C (n = 8/group). The female offspring were monitored until 24 wk of age in terms of body weights (from 1 wk of age onward to avoid maternal rejection of the pups) and food intake (from weaning). Systolic blood pressure (SBP), biochemical markers (total cholesterol [TC], LDL-C, and high-density lipoprotein cholesterol [HDL-C]) and CRP, and colonies of the bone marrow mononuclear cells were measured up to 24 wk.

Study part 2

In this part of the study, 50% of the HF-fed females were given the water-soluble pravastatin (Sigma UK; 5 mg/kg daily) in their drinking water. These females were fed the assigned diet and treated with statin through weaning to pregnancy and lactation. After birth, the pups were weighed and litter size was reduced to eight female pups. From weaning (21 d postpartum), offspring from the HF and HF+ S dams were fed the same HF diet, and were referred to as the HF/HF and HF+S/HF dietary groups, respectively (n = 8/group). Offspring from the dams fed the same diet postweaning were referred to as the C/C group (Fig. 1). Colonies of bone marrow-derived mononuclear cells were measured up to 24 wk.