Biochimica et Biophysica Acta 1862 (2016) 113–120

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Leptin dose-dependently decreases atherosclerosis by attenuation of hypercholesterolemia and induction of adiponectin

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ARTICLE INFO

Article history:
Received 25 August 2015
Received in revised form 8 October 2015
Accepted 17 October 2015
Available online 28 October 2015

Keywords:
Adiponectin
Adipokine
Atherosclerosis
Insulin resistance
Leptin
Obesity

ABSTRACT

Objectives: Conflicting evidence concerning leptin in atherosclerosis has been published. Furthermore, dose-dependent effects of leptin on atherogenesis have not been studied.
Methods: Leptin-deficient low-density lipoprotein receptor (LDLR) knockout (LDLR$^{-/-}$;*ob*/*ob*) mice were treated with saline, 0.1, 0.5, or 3.0 mg/kg body weight (BW)/d recombinant leptin over 12 weeks starting at 8 weeks of age. Aortic root and brachiocephalic artery (BCA) atherosclerotic lesions were analyzed by oil red O staining. Furthermore, glucose homeostasis, lipid metabolism, and liver function including tissue studies were assessed in all animals.
Results: Leptin treatment dose-dependently decreased BW in LDLR$^{-/-}$;*ob*/*ob* mice as compared to saline. Mice in the 0.1 and 0.5 mg/kg BW/d groups remained heavier (i.e. subphysiological leptin dose) and in the 3.0 mg/kg BW/d group had similar weight (i.e. physiological leptin dose) as compared to non-leptin-deficient LDLR$^{-/-}$ animals. Recombinant leptin dose-dependently reduced plaque area in the aortic root and the BCA by 36% and 58%, respectively. Leptin-mediated reductions of plasma total and LDL-cholesterol (Chol) remained independent predictors for aortic root plaque area. Chol content in liver, as well as hepatic expression of key lipid and proinflammatory genes, were dose-dependently regulated by leptin. Furthermore, leptin treatment increased circulating levels and adipose tissue mRNA expression of the adipokine adiponectin.
Conclusions: Leptin administration within the subphysiological to physiological range diminishes atherosclerotic lesions. Leptin appears to mediate its antiatherogenic effects indirectly through reduction of hypercholesterolemia and liver steatosis, as well as upregulation of insulin-sensitizing and atheroprotective adiponectin.

1. Introduction

Atherosclerosis is a lipid-driven chronic inflammatory disease and the main cause of cardiovascular morbidity and mortality. It has been recognized that obesity which is associated with an expansion of adipose tissue is a predisposing risk factor for atherosclerosis [1]. Adipose tissue has pleiotropic effects beyond energy storage and functions as an endocrine organ that secretes various bioactive molecules [2]. Proteins that are secreted by adipose tissue and that provide an extensive network of para- and endocrine communication are collectively called adipokines [3].

Among those, leptin is a 16 kDa adipokine which is dysregulated in obesity and influences appetite and body weight (BW) [4,5]. The adipokine is transported across the blood–brain barrier where it binds to specific receptors of appetite-modulating neurons most notably in the hypothalamus [6]. In accordance with an appetite-suppressive effect, leptin-deficient *ob*/*ob* mice, as well as human subjects, develop severe hyperphagia and excessive morbid obesity associated with dyslipidemia, fatty liver disease, insulin resistance (IR), hyperinsulinemia, and diabetes mellitus type 2 [7,8]. Administration of recombinant leptin in leptin-deficient mice and humans reduces food intake, BW, and fat mass [9,10].

Whereas leptin has been well-established as an appetite-suppressive adipokine, its role in vascular disease is far from clear since conflicting data have been published. Thus, various independent studies suggest that the adipokine has antiatherogenic properties. Among those, Lloyd and co-workers have shown convincingly that leptin treatment ameliorates atherosclerotic disease in atherosclerosis-prone apolipoprotein (Apo) E triple-knockout- (ApoE$^{-/-}$;ApoB48$^{-/-}$;*ob*/*ob*) and low-density lipoprotein receptor (LDLR) triple-knockout (LDLR$^{-/-}$;ApoB48$^{-/-}$;*ob*/*ob*) mice [11]. Furthermore, leptin inhibited the progression of atherosclerosis in type 1 diabetic ApoE$^{-/-}$;Ins2$^{+/Akita}$ mice by attenuating hypercholesterolemia [12]. In addition,

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http://dx.doi.org/10.1016/j.bbadis.2015.10.022
0925-4439/

leptin-deficiency in LDLR knockout (LDLR$^{-/-}$;*ob*/*ob*) mice resulted in accelerated IR-associated atherosclerosis and dyslipidemia as compared to LDLR$^{-/-}$ controls [13,14]. In contrast to these findings, leptin treatment increased atherosclerosis and shortened the time to occlusive thrombosis after vascular injury [15]. Similarly, Zeadin and co-workers convincingly demonstrated that leptin-treated animals were characterized by an increase in lesion calcification, as well as valvular calcification, as compared to vehicle-treated controls [16]. Furthermore, daily administration of recombinant leptin to *ob*/*ob* mice over 3 weeks after carotid artery injury with ferric chloride dramatically increased neointimal thickness and the severity of luminal stenosis [17].

Taking these studies into consideration, leptin appears to have both pro- and antiatherogenic effects which might depend on the dose of the adipokine used. However, to the best of our knowledge dose-dependent effects of leptin on atherogenesis have not been assessed so far. Furthermore, it remains unclear which metabolic parameters predict the impact of the adipokine on vascular disease. To address these open points, atherosclerosis-prone and leptin-deficient LDLR$^{-/-}$;*ob*/*ob* mice were treated in the current study with three increasing fixed doses of recombinant mouse leptin. Leptin doses ranged from subphysiological levels, i.e. doses not sufficient to normalize BW, to physiological levels, i.e. doses sufficient to normalize BW to LDLR$^{-/-}$ control levels. We hypothesized that within this subphysiological to physiological range, leptin has potent antiatherogenic effects in LDLR$^{-/-}$;*ob*/*ob* mice which are mediated through improved metabolic function.

2. Materials and methods

2.1. Animal care

The local ethics committee (Landesdirektion Sachsen, Leipzig) of the state of Saxony approved the protocol of all animal experiments (approval no. TVV37/12). Breeding and husbandry of homozygous male LDLR knockout and leptin-deficient (LDLR$^{-/-}$;*ob*/*ob*) mice was done in our local animal facility, the Medical Experimental Center of the University of Leipzig. All mice were maintained in a room under pathogen-free conditions with controlled 21 ± 1 °C on a 12:12 h light/dark cycle (6 AM/6 PM). Water and chow pellet diet (V1534 from Sniff, Soest, Germany) was provided ad libitum until age of 4 weeks.

2.2. Animal treatment

LDLR$^{-/-}$;*ob*/*ob* mice were fed ad libitum with a modified, cholesterol (Chol)-enriched semisynthetic Clinton/Cybulsky diet (Sniff, Soest, Germany) starting at the age of 4 weeks. At 8 weeks of age, body weight (BW)-matched mice were separated into four groups and treated daily over a period of 12 weeks i.p. with saline or increasing concentrations of recombinant mouse leptin (0.1 mg/kg BW, 0.5 mg/kg BW, and 3.0 mg/kg BW) (R&D Systems, Wiesbaden—Nordenstadt, Germany). At 20 weeks of age, mice were sacrificed by exsanguination under deep anesthesia.

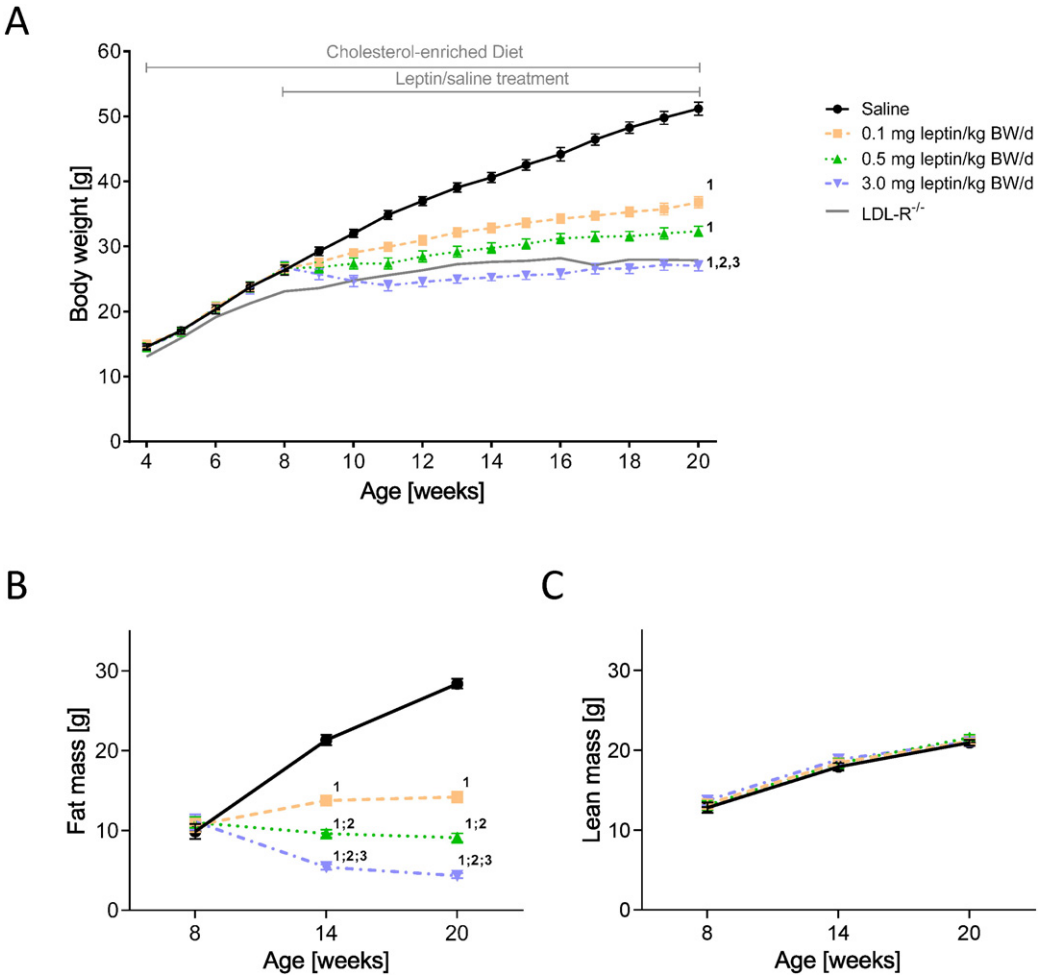

Fig. 1. (A) BW (N ≥ 19 per group LDLR$^{-/-}$;*ob*/*ob*; N = 8 LDLR$^{-/-}$), (B) fat mass (N ≥ 9 per group), and (C) lean mass (N ≥ 9 per group) over the course of treatment in leptin- and saline-treated LDLR$^{-/-}$;*ob*/*ob* mice. Statistical analysis was performed by one-way ANOVA of (A) AUC or (B, C) raw data corrected for multiple testing by Bonferroni–Holm. Data are presented as means ± standard error. $P < 0.05$ as compared to [1]saline; [2]0.1 mg leptin/kg BW/d; [3]0.5 mg leptin/kg BW/d.