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Short Communication

A high fat diet does not stimulate blood pressure dependence on chemerin in the Sprague-Dawley rat

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ABSTRACT

The adipokine chemerin is a candidate for connecting obesity to hypertension.
Study objective: To test the hypothesis that a high fat (HF) diet stimulates dependence on chemerin for blood pressure regulation.
Design: Blood pressure in male Sprague Dawley rats fed a control (10 % fat) or HF (60 % fat) diet from weaning was measured using radiotelemetry. Antisense oligonucleotides (ASOs), administered after 17 weeks of feeding, were used to abolish chemerin production.
Results: The HF diet did not increase blood pressure (mm Hg; control = 117.0 ± 2.5; HF = 122.0 ± 2.2). An ASO against chemerin (dosed 1×/week, 4 weeks) similarly reduced blood pressure in the control (−14.0 ± 2.7 mmHg) and HF rat (−12.4 ± 2.3). Chemerin mRNA was abolished in the liver and fats (primary producers of chemerin) from rats given the ASO chemerin *vs* control.
Conclusion: A HF diet alone is insufficient to stimulate the dependence of blood pressure in the rat on chemerin.

1. Introduction

Because obesity is defined as an increase in fat burden, adipokines (substances made within the fat) are likely at least partially causal for the pathology of obesity-associated hypertension (https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight). The adipokine chemerin is a leading candidate because of a positive association of circulating levels of chemerin with body mass index (BMI), visceral fat burden, systolic and diastolic blood pressure, and/or arterial stiffness in multiple human populations and animal models of cardiovascular (CV) disease [1,2]. In basic studies, chemerin is largely pro-hypertensive in its actions to cause direct vascular contraction, indirect vascular contraction (amplification of electrical field stimulated-induced contraction) and smooth muscle mitogenesis [1]. These actions are carried out primarily by the G protein coupled Chemerin1 receptor [1]. Consistent with these pro-hypertensive vascular functions, use of an antisense oligonucleotide (ASO) that destroys chemerin mRNA (and thus reduces chemerin protein expression) reduced the blood pressure of the Dahl SS rat on a high fat (HF) feeding from weaning [3]. On this model of elevated adiposity, knockdown of chemerin reduced mean arterial blood pressure over 30 mmHg [4]. We hypothesized that the ASO reduced blood pressure by removing chemerin, elevated by increased fat burden.

This led to the important question of whether a HF diet *stimulated* the dependence of blood pressure on chemerin. This is logical given that a greater fat burden provides greater amounts of chemerin. Presently, we test this idea by using the same HF diet protocol used in the Dahl SS experiment described above but in the Sprague Dawley rat, a model that gains significant fat with HF diet. Using radiotelemetry to measure blood pressure, we test the hypothesis that, as with the Dahl SS rat, HF feeding from weaning supports a blood pressure that is more chemerin dependent *vs* a normal diet. This would be evidenced by a greater fall in the blood pressure in the HF fed animals *vs* control fed rats upon administration of the ASO that prevents chemerin expression.

2. Methods

2.1. Animal use

Male Sprague Dawley rats (from weaning, ~3–4 weeks of age; Charles River, Indianapolis, IN, USA) were used following the Guide for

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the Care and Use of Laboratory Animals (8th edition, 2011). Rats were fed a control Diet (Control at 10 % fat; Diet D12450J, Research Diets, New Brunswick, NJ, USA) or high fat diet (HF at 60 % fat; Diet D12492, Research Diets) for 17 weeks prior to beginning the injections series described below.

Only one Control and HF SD rat given vehicle was carried along given that our primary purpose was to determine how the ASO reduced the blood pressure of the Control and HF relative to one another. Vehicle injected rats serve as a comparator. Additionally, a scrambled ASO sequence of the same length and backbone as the ASO against chemerin has no effect on blood pressure [4]. Thus, our focus is on the ASO against chemerin. The Michigan State University Institutional Animal Care and Use Committee (IACUC) guidelines (Protocol # 02–18-026, approved 18 February 2018) were followed for all protocols.

2.2. Telemetry implantation

Radiotelemeter transmitters (model TA11PA C40 or S10; Data Sciences International, MN USA) were implanted as previously published and at approximately after 5 weeks on diet [4]. Measures were recorded for 10 s every 10 min throughout the duration of the study and are presented as a 24-h average as collected by the DataQuest A.R.T. Version 4.2 software.

2.3. ASO against chemerin administration

The ASO was synthesized by IONIS Pharmaceuticals (Carlsbad, CA, USA) with the following sequence: ASO Gen 2.5 (5′-3′): GTTTTATTAGCCTGGA. ASO solution was confirmed for concentration and purity using a Nanodrop 2.0. ASOs were dissolved in phosphate buffer saline (PBS). Both ASO (25 mg/kg) and vehicle (volume control) were injected subcutaneously while animal was under light isoflurane anesthesia (2 %, balance oxygen).

2.4. Tissue dissection and RT-PCR

Pentobarbital (80 mg/kg ip) was used to anesthetize rats and a bilateral pneumothorax created. Liver, epididymal fat, retroperitoneal fat, mesenteric fat and thoracic aortic perivascular adipose tissue (PVAT) and spleen were dissected from the rat. Adipose tissue immediately surrounding an artery (mesenteric, aortic) was considered its PVAT. Under a stereomicroscope and using microscissors, PVAT was removed from vessels (APVAT from aorta) in a buffer (135 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L K_2HPO_4, 5.5 mmol/L glucose, 20 mmol/L HEPES, 10 mL antibiotic/antimycotic, pH to 7.4). Samples were prepared for real time polymerase chain reaction (RT-PCR) as previously described [4]. Measures were compared by running the housekeeping gene *β-actin*. Primers were obtained from Integrated DNA Technologies (IDT; Coralville, IA, USA) and were as follows:

rRarres2 (chemerin) (306118822)
Primer 1: GAGCTTAAATTCCAGCCTCACAA
Primer 2: CAGGAGATCGGTGTGGACAGT
Probe: /56-FAM/TGATGACCTGTTCTTCTCAGCTGGCACC/36-TAMSp/

β-actin (306118826)
Primer 1: TCACTATCGGCAATGAGCG
Primer 2: GGCATAGAGGTCTTTACGGATG
Probe: /56-FAM/TCCTGGGTATGGAATCCTGTGGC/36-TAMSp/

2.5. Data presentation and analyses

All values reported represent means ± SEM for the number of biological replicates (*n*). Student's *t*-test was used when comparing two groups and a One-way ANOVA with a Tukey correction when comparing more than two groups. A $p < 0.05$ was considered statistically significant. All data were graphed and analyzed statistically using Graph Pad Prism (GraphPad Software, San Diego, CA, USA, RRID: SCR_002798).

3. Results

Table 1 provides measures of final body weight and organ weights for rats dissected two (2) days after the last vehicle/ASO injection. RP and Epi fat were statistically greater (absolute, as a % body weight) in the HF-fed *vs* Control-fed diet though body weights of the HF diet fed rats on the ASO were not statistically different from Control. Liver weight as a % of body weight was reduced in the HF *vs* Control.

3.1. HF diet did not cause hypertension in the Sprague Dawley rat

Fig. 1A shows that in the five days prior to the first vehicle/ASO injection, the mean arterial blood pressure of the groups to be injected with ASO (Control and HF) were not statistically different (repeated measures ANOVA, $p > 0.05$). Notably, the HF diet had not elevated the blood pressure of the HF group above that of the Control group.

3.2. ASO against chemerin reduced blood pressure of control and HF rats by similar magnitude

Fig. 1B shares the 24-h averaged mean arterial blood pressure immediately prior to and after each of the four vehicle/ASO injections. The blood pressure of both groups fell compared to their own baseline (in key), but the magnitude of the greatest fall from their baseline between the Control (−14.0 ± 2.7 mmHg) and HF groups (−12.4 ± 2.3 mmHg) receiving the ASO was not statistically different ($p < 0.05$).

3.3. ASO against chemerin effectively abolished chemerin mRNA expression in all tissues

The ASO against chemerin was effective in reducing chemerin mRNA (*Rarres2)* gene expression, the intended target of the ASO, in all tissues (Fig. 2). When comparing the Control and HF group that received the ASOs, final chemerin expression (zero in most cases) was not statistically different. Plasma chemerin, measured by Western analyses, was abolished in all rats that received the ASO (not shown).

4. Discussion

These findings provide the important evidence that a high fat (HF) diet alone is insufficient to cause blood pressure to become more dependent on chemerin for maintenance/elevation of blood pressure.

Table 1
Final phyiological parameters of the four experimental groups of Sprague Dawley rats.

Measure	Control	Control ASO	HF	HF ASO
Body weight (grams)	644	588 ± 17	919	628 ± 34
Liver weight (grams)	21.7	20.0 ± 0.5	30.4	19.4 ± 1.5
Liver weight/body weight (%)	3.4	3.4 ± 0.1	3.3	3.1 ± 0.1*
Retroperitoneal fat weight (grams)	23.0	15.6 ± 1.8	78.2	26.8 ± 3.5*
Retro fat weight/body weight (%)	3.6	2.70 ± 0.3	8.5	4.24 ± 0.4*
Epididymal fat weight (grams)	11.0	9.7 ± 1.1	20.7	13.4 ± 1.0*
Epi fat weight/body weight (%)	1.7	1.6 ± 0.2	2.2	2.1 ± 0.1*
Spleen weight (grams)	1.1	2.3 ± 0.3	1.3	2.3 ± 0.3
Spleen weight/body weight (%)	0.16	0.39 ± 0.05	0.15	0.36 ± 0.03
Heart Weight (grams)	1.70	1.7 ± 0.07	2.68	2.0 ± 0.11
Heart weight/body weight (%)	0.26	0.30 ± 0.01	0.29	0.32 ± 0.02

• = statistical significance *vs* Control ASO values ($p < 0.05$, two way Students *t*-test).

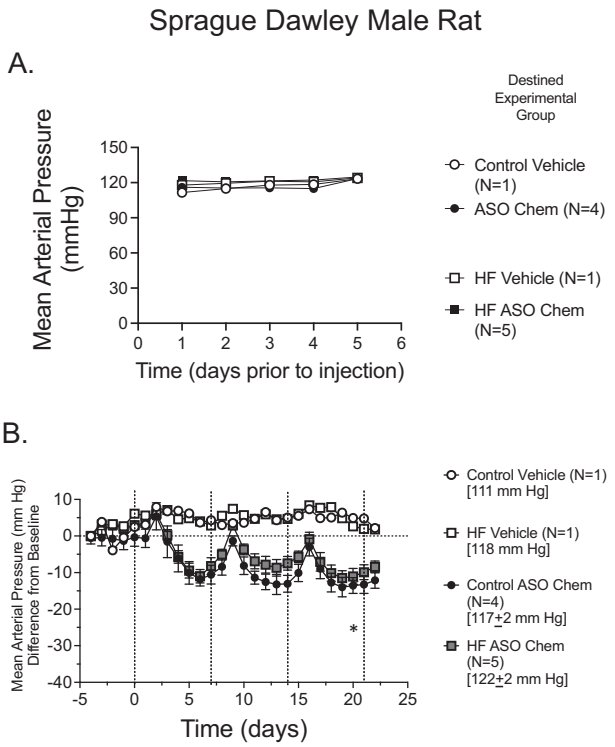

Fig. 1. A. Baseline mean arterial blood pressure, measured telemetrically, in the four experimental groups. Points are means+SEM for the number of animals indicated in parentheses. B. Fall in mean arterial blood pressure, from baseline, in groups given vehicle or weekly injection of ASO directed against chemerin. Dotted vertical lines indicate time of injection. Points are means+SEM for number of animals indicated in parentheses. Key also includes the mean arterial blood pressure of groups immediately prior to first injection. * signifies statistically significant difference from time 0 blood pressure.

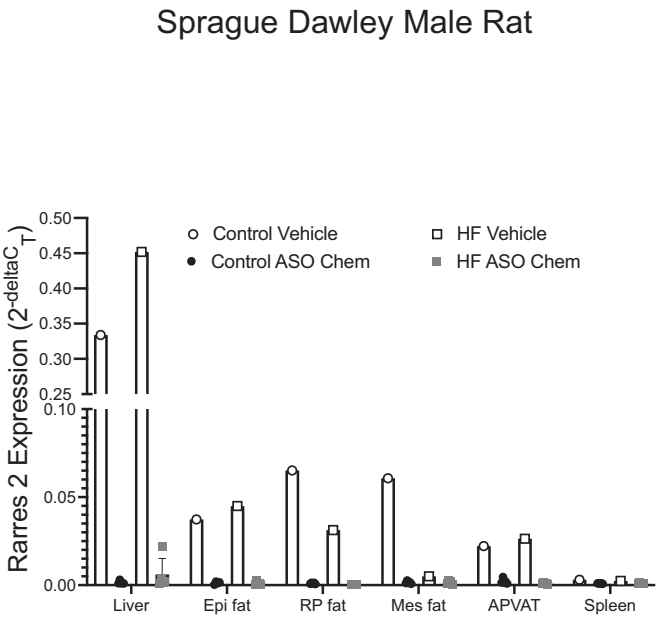

Fig. 2. Chemerin mRNA (Rarres2) expression in tissues [liver, epididymal fat, retroperitoneal (RP) fat, mesenteric fat, aortic PVAT (APVAT) and spleen] taken two days after final vehicle/ASO injection. Bars represent means ± SEM with individual points scattered.

The ASO against chemerin reduced the mean arterial blood pressure of both the Control and HF diet fed Sprague Dawley rat by equivalent magnitudes.

We interpret this to mean that the Sprague Dawley rat is unlike the Dahl SS rat in the chemerin/chemerin receptor axis. These two strains of rats, when fed the same HF diet from weaning, have a categorically different blood pressure response. The Dahl SS rat develops a hypertension while the Sprague Dawley rat does not [3]. The present experiment was important for two reasons. First, we could reproduce the lack of a hypertensive experienced by the Sprague Dawley rat with a HF diet from weaning, though the Sprague Dawley rat gained fat. Second, while the Sprague Dawley rat did not develop a hypertension with the HF diet, the dependence on chemerin for control of blood pressure could have been changed by the diet. Our data support that this is not the case. In the present study, we adopted a standard protocol for use of ASOs which uses weekly injections for four weeks. Though not shown, one injection of the ASO Chem is sufficient to reduce liver and fat chemerin protein by 50 % in the rat 48 h after injection. These data support chemerin is made constitutively at some level and that the ASO against chemerin would work acutely.

These findings raise several important questions as to why the blood pressure of two strains of rats respond so differently to a HF diet. If the burden of fat does not matter, then what does? Two possibilities can be considered. First, chemerin isoforms exist and these do not possess equivalent biological activity [5]. We speculate that the tissues of the Dahl SS make a greater amount of active chemerin isoforms (Chem 157 isoform) *vs* the inactive isoforms (Chem 163 isoform). Research in human obesity supports that a majority of circulating chemerin is inactive and active isoforms may increase in a tissue-specific way [5,6] including production of unattributed chemerin forms [7]. Antibodies towards specific forms of chemerin within the rat are not available to address these ideas within this model. Second, there may be a greater expression or sensitivity of chemerin receptors expression, specifically Chemerin1, in tissues/organs that regulate blood pressure in the Dahl SS *vs* the Sprague Dawley rat. Studying this idea, at least at the receptor level, is problematic because antibodies to detect and quantify G protein coupled receptors, a class to which all chemerin receptors belong, are non-specific.

What do these findings mean for human health? Chemerin should be considered a *bona fide* target for hypertension therapeutics and studies like the present help continually refine the forms of hypertension in which chemerin is most importantly studied. Here we demonstrate the potential utility and effectiveness of a drug that inhibits the ability of chemerin protein ultimately being formed.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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