Original Article

MOLECULAR METABOLISM

# Adropin: An endocrine link between the biological clock and cholesterol homeostasis

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## ABSTRACT

**Objective:** Identify determinants of plasma adropin concentrations, a secreted peptide translated from the *Energy Homeostasis Associated* (*ENHO*) gene linked to metabolic control and vascular function.

**Methods:** Associations between plasma adropin concentrations, demographics (sex, age, BMI) and circulating biomarkers of lipid and glucose metabolism were assessed in plasma obtained after an overnight fast in humans. The regulation of adropin expression was then assessed *in silico*, in cultured human cells, and in animal models.

**Results:** In humans, plasma adropin concentrations are inversely related to atherogenic LDL-cholesterol (LDL-C) levels in men ($n = 349$), but not in women ($n = 401$). Analysis of hepatic *Enho* expression in male mice suggests control by the biological clock. Expression is rhythmic, peaking during maximal food consumption in the dark correlating with transcriptional activation by ROR$\alpha/\gamma$. The nadir in the light phase coincides with the rest phase and repression by Rev-erb. Plasma adropin concentrations in nonhuman primates (rhesus monkeys) also exhibit peaks coinciding with feeding times (07:00 h, 15:00 h). The ROR inverse agonists SR1001 and the 7-oxygenated sterols 7-β-hydroxysterol and 7-ketocholesterol, or the Rev-erb agonist SR9009, suppress *ENHO* expression in cultured human HepG2 cells. Consumption of high-cholesterol diets suppress expression of the adropin transcript in mouse liver. However, adropin over expression does not prevent hypercholesterolemia resulting from a high cholesterol diet and/or LDL receptor mutations.

**Conclusions:** In humans, associations between plasma adropin concentrations and LDL-C suggest a link with hepatic lipid metabolism. Mouse studies suggest that the relationship between adropin and cholesterol metabolism is unidirectional, and predominantly involves suppression of adropin expression by cholesterol and 7-oxygenated sterols. Sensing of fatty acids, cholesterol and oxysterols by the ROR$\alpha/\gamma$ ligand-binding domain suggests a plausible functional link between adropin expression and cellular lipid metabolism. Furthermore, the nuclear receptors ROR$\alpha/\gamma$ and Rev-erb may couple adropin synthesis with circadian rhythms in carbohydrate and lipid metabolism.



**Keywords** Cholesterol; LDL; Cardiovascular disease; Obesity; Adropin; Sex

## 1. INTRODUCTION

Secreted peptides are involved in signaling metabolic status at the organismal and cellular levels to maintain cardiovascular and metabolic homeostasis. Studies in male C57BL/6J (B6) mice suggest the secreted peptide adropin provides such a signal of metabolic condition [1]. Adropin is a product of the *Energy Homeostasis Association* (*ENHO*) gene, comprised of two-exons on human chromosome 9p13.3. A highly conserved open reading frame in exon 2 encodes the full-length peptide (adropin$^{1-76}$). Adropin$^{1-33}$ is a secretory signal peptide [1]; adropin$^{34-76}$ is biologically active when administered to mice and cultured cells. For example, adropin$^{34-76}$ alters whole body glucose and lipid metabolism when administered to mice [1,2], rats [3,4], and also activates signaling pathways in mammalian cell lines [5]. Adropin$^{34-76}$ may also function to preserve the circulatory system, regulating endothelial function and activity of endothelial nitric oxide

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synthase [5–8]. The *Enho* transcript is however widely expressed, with high levels of expression in the nervous system relative to other tissues in B6 mice [1,5,9]. Analysis of male B6 mice that are adropin-deficient, over express adropin or are administered pharmacological doses of synthetic peptide suggest adropin suppresses fat oxidation and enhances oxidative glucose disposal and glucose tolerance [1,2,10].

Studies investigating plasma adropin concentrations in humans and nonhuman primates have observed associations with diet [11–14], with indices of insulin resistance [6,11,15,16], and with risk for cardiovascular disease [17,18]. However, determinants of plasma adropin concentration in humans are still poorly defined.

Here we report that plasma adropin concentrations are inversely related to plasma levels of low-density lipoprotein cholesterol (LDL-C) in males, but not in females. We also report that the biological clock may be plausible focal point linking *Enho* transcription with nutrient intake and cellular metabolic condition.

## 2. MATERIALS AND METHODS

### 2.1. Human studies

Plasma adropin concentrations were measured in the EDTA plasma fraction of blood samples collected after an overnight fast in five studies:

- 389 participants of a previously unpublished and unregistered Reference Range Study (RRS);
- Two NIH funded studies examining the impact of sugar consumption over 2 wk (n = 182) (DRS, NCT01103921) [19] or 10 wk (n = 31) (IPOP, NCT01165853) [20] on cardiometabolic risk factors;
- A randomized intervention trial examining weight loss on cardiometabolic risk factors (Caloric Restriction, Exercise, and Glucoregulation in Humans; NCT00777621) (n = 68) [21]; and
- Samples from participants of the HERITAGE study (NCT00005137) examining the interactions between genetics and exercise on cardiometabolic risk factors (n = 80) [22].

The relationship between plasma adropin concentrations and atherogenic cholesterol was assessed in data pooled from the RRS, DRS, IPOP, CREG and HERITAGE studies [11,12,15]. These data sets were selected based on collection of common demographic and blood chemistry data (**supplemental data**, Table S1). The studies are cross sectional, using plasma samples collected at baseline prior to dietary and/or behavioral interventions, and incorporated previously published data from smaller studies [11,12,15].

#### 2.1.1. Reference range study

The original study involved 395 participants (190 men, 205 women) aged 21–75y recruited by investigators at the University of California, Davis, CA. EDTA-plasma samples were available from 187 male and 202 female volunteers for a study designed to validate a commercial assay for small diameter LPL. Study participants were recruited through an Internet listing (craigslist.com) and local postings of flyer. Subjects aged <21 and >75 years, used drugs of abuse, used drugs affecting lipoprotein metabolism (statins, fibrates, bile acid sequestrants or nicotinic acid), taking drugs for treating diabetes, are on hormone replacement therapy, had current or recent cardiovascular/coronary heart disease, or with current or recent cancer were excluded. Body mass index (BMI) ranged from 16.3 to 43 kg/m$^2$ (11 underweight), BMI <18.5 kg/m$^2$; 210 normal/healthy body weight, BMI 18.5–24.9 kg/m$^2$; 113 overweight (BMI 25.0–29.9 kg/m$^2$; 55 obese, BMI > 30 kg/m$^2$). The study was conducted in accordance with an experimental protocol reviewed and approved by the UC Davis Institutional Review Board. Participants provided written informed consent.

#### 2.1.2. Dietary sugar studies

Participants in these studies are subgroups of NIH-funded investigations involving 182 participants (DRS) [19] and 31 participants (IPOP) [23,24]. DRS participants (92 men, 90 women) were recruited through internet listings (craigslist.com) and local postings of flyers, underwent telephone and in-person interviews with medical history, complete blood count, and serum biochemistry panel to assess eligibility. Inclusion criteria included age 18–40 y and BMI 18–35 kg/m$^2$ with a self-report of stable body weight during the prior 6 months. Exclusion criteria included diabetes (fasting glucose >125 mg/dL), evidence of renal or hepatic disease, fasting plasma triglyceride >400 mg/dL, hypertension (>140/90 mm Hg), hemoglobin <8.5 g/dL, and surgery for weight loss. Individuals who smoked, habitually ingested >2 alcoholic beverages/d, exercised >3.5 h/wk at a level more vigorous than walking, or used thyroid, lipid-lowering, glucose-lowering, antihypertensive, antidepressant, or weight loss medications were also excluded. The study was conducted in accordance with an experimental protocol that was approved by the UC Davis Institutional Review Board, and participants provided written informed consent.

Participants for IPOP were recruited through newspaper advertisements and underwent a telephone and an in-person interview with medical history, a complete blood count, and a serum biochemistry panel to assess eligibility. Inclusion criteria included age from 40 to 72y and BMI of 25–35 kg/m$^2$ with a self-report of stable body weight during the prior 6 months. Women were considered postmenopausal based on a self-report of no menstruation for at least 1 year. Exclusion criteria included evidence of diabetes, renal disease, or hepatic disease; fasting serum TG concentrations greater than 400 mg/dL; hypertension (>140/90 mmHg); and history of surgery for weight loss. Individuals who smoked, reported exercise of more than 3.5 h/wk at a level more vigorous than walking, or reported having used thyroid, lipid-lowering, glucose-lowering, antihypertensive, antidepressant, or weight-loss medications were also excluded. Diet-related exclusion criteria included habitual ingestion of more than 1 sugar-sweetened beverage per day or more than 2 alcoholic beverages per day. The UC Davis Institutional Review Board approved the experimental protocol, and subjects provided informed consent for participation in the study.

#### 2.1.3. CREG study

Selection criteria, interventions, and outcomes were reported previously [11,21]. The study involved sedentary, men and women with excess weight aged 45–65y. The study was reviewed and approved by the Institutional Review Boards (IRB) of Washington University and Saint Louis University.

#### 2.1.4. HERITAGE study

Plasma from 40 participants (20 males, 20 females) showing no response ("nonresponders") and 40 participants (20 males and 20 females) exhibiting high training-induced gains in $VO_2$max ("responders") were selected by ranking participants from minimal to maximal response, stratified by sex. The current study uses baseline data.

#### 2.1.5. Blood chemistries and measurement of circulating hormones

For the RRS, lipids were measured on a Hitachi 911 analyzer (Hitachi Inc, Tokyo, Japan) at Tufts University. For the DRS and IPOP, lipid

 