Effect of Experimental Obesity and Subsequent Weight Reduction upon Circulating Atrial Natriuretic Peptide (42932)

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Abstract. **The effect of obesity and weight reduction upon circulating concentrations of atrial natriuretic peptide was assessed in an experimental model of the disease. Obese rats weighing in excess of 750 g were compared with formerly obese animals subjected to a 15-week period of caloric restriction resulting in a 40% reduction in body weight. Mean adipocyte size was significantly reduced with weight loss, as was estimated body fat. Mean arterial blood pressure remained normotensive for both groups, but a significant reduction in heart rate was associated with weight reduction. Circulating atrial natriuretic peptide was significantly elevated in the lean rats, which also exhibited decreased plasma renin activity and a negative sodium balance. Analysis of heart to body weight ratios implied that an obesity-associated, volume-induced cardiac hypertrophy remained even after the normalization of body fat. These results suggest that the diuresis and natriuresis accompanying weight reduction may be facilitated by atrial natriuretic peptide, which was elevated in part due to a persistent left ventricular hypertrophy following the transition from the obese to lean condition.**

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From a cardiovascular perspective, obesity has been described as a disease of volume expansion, exemplified by an increased blood volume, plasma volume, and stroke volume (1, 2). The vascular response to this volume expansion is variable, however, and may or may not be associated with hypertension. The standard treatment of obesity is weight reduction, and a variety of nonpharmacologic regimen have been utilized in reaching this end point. Regardless of the method employed, the underlying basis of the necessity for weight loss is the alleviation of the aforementioned volume expansion, the composition of which can be predominantly water (3). In fact, experimental studies have shown that water content of adipose tissue increases significantly with obesity (4). Therefore, while the basis of the variable hemodynamic response to obesity remains obscure, the development of cardiovascular symptoms is due largely to an expanded blood volume, and treatment necessarily involves significant reversal of this situation either acutely through drug-induced diuresis or chronically through caloric restriction (5).

The relatively recent discovery of atrial natriuretic peptide and its involvement in fluid homeostasis has established the heart as an endocrine organ (6). Briefly, secretion of the atrial peptide is primarily dependent upon atrial distention and results in a reduction of blood pressure, increased diuresis, and natriuresis (7, 8). Interestingly, although obese humans exhibit increased intravascular volume, enlarged atria, and oftentimes elevated atrial pressures (9), an investigation of the physiology of atrial peptides in the obese state has not been performed. This study was designed to determine the relationship between circulating rat atrial natriuretic peptide, hemodynamics, and adipose tissue mass both in experimental obesity and during the transition from the obese to lean state.

Materials and Methods

Spontaneously obese rats were obtained from a colony maintained at American Cyanamid Co. These rats were originally obtained from Charles River Breeding Laboratories (Wilmington, MA), were of the Sprague-Dawley strain, and developed obesity through excessive caloric intake and relative inactivity (10). This is therefore a model of dietary obesity, as opposed to

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experimental models of genetic or brain-lesion-induced obesity.

Obese rats, 9 months old, were initially divided into two groups of equal body weight of approximately 750 g. One group was selected to receive a calorically restricting diet of 30–40% *ad libitum* intake of the obese rats, which resulted in an initial rapid decline in body weight, followed by weight maintenance. Rats remained on either the restricted diet or an *ad libitum* control diet for a 15-week period, the end point of which was preparation for hemodynamic monitoring.

Rats were prepared for hemodynamic monitoring by placement of a saline-filled cannula composed of PE 10 tubing into the tail artery. Preoperative preparation initially involved restraint of the rats in a standard apparatus, followed immediately by a subcutaneous injection of 0.25 ml of 2% procaine into the tail. A 1-cm incision was then made at the base of the tail, and the artery was exposed, cannulated, the incision closed, and the wound was covered with a surgical dressing. The entire procedure was completed in approximately 15 min. The rats were subsequently placed in standard plastic restraining cages for hemodynamic monitoring. A 30-min period of acclimation elapsed before hemodynamic monitoring began. Each cannula was connected to a transducer and physiologic recorder, which was interfaced to a computer. Data were acquired and stored to disk at 100 samples/sec and updated every 10 sec. Accumulation of values for mean arterial blood pressure, as well as heart rate, were stored every 15 min, and those hemodynamic values obtained at the end of a 1-hr recording period were considered as representative of the resting hemodynamics of each rat. Additional details of these procedures have been described previously by our group (10, 11).

Immediately following the hemodynamic monitoring period, the rat was separated from the recording apparatus and 1 ml of arterial blood was allowed to flow freely into collecting tubes containing 20 KIU of aprotinin, which was subsequently spun at 3000 rpm for 20 min. The cannulas were sealed and rats returned to their cages. Plasma was stored at −70°C for not more than 1 week, then analyzed for α-rat atrial natriuretic peptide using a commercially available radioimmunoassay procedure (Peninsula Labs, Belmont, CA). Before analysis, all samples were coded in triplicate and then decoded by an observer only after final calculations were completed. Rats were then killed with a guillotine, cavity blood was collected, hearts were quickly excised, and atrial and ventricular weights were determined using an analytical balance. Left ventricular thickness was determined with three separate measurements from the midline of the left ventricle using a microprocessor controlled digital caliper.

Sodium balance was determined by placing rats not used for hemodynamic monitoring into metabolic cages. Water intake and urine volume over a 24-hr period were assessed on a daily basis during the final 2 weeks of the 15-week study. Obese rats were fed *ad libitum* during this period, while weight-reduced rats were given a maintenance diet. Food was provided as Purina Rat Chow #5002 (St. Louis, MO), which contained by weight 0.30% sodium, 0.86% potassium, and 0.47% chlorine. Sodium intake was then calculated by multiplying food intake by sodium content. Urine sodium concentration was determined with an ion-selective electrode, in combination with appropriate standards. The total sodium excreted in the urine was then calculated by multiplying the sodium concentration by the total urine volume.

Plasma renin activity (ng/ml/hr) was assessed using a commercially available radioimmunoassay procedure (Baxter Healthcare Corp., Cambridge, MA). Blood was collected in tubes containing EDTA, centrifuged, and plasma stored at −65°C prior to the assay. Control values obtained were within 0.25 ng/ml/hr of label information provided by the supplier.

For estimating body fat, the epididymal depot adjacent to each testis was removed, rinsed free of blood, blotted dry, and weighed. A section weighing approximately 250 mg was then dissected from the depot and incubated for 1 hr at 37°C in a solution of Krebs-Ringer bicarbonate (pH 7.4) containing 4% albumin and collagenase at a concentration of 2 mg/ml. The resulting fat cell digest was passed through a 149-μm mesh screen, and the isolated cells were washed twice with fresh medium without collagenase, resuspended, and fixed in 2% glutaraldehyde. Within 1-week postfixation, the diameter of 200 cells was determined using a microscope with a calibrated eyepiece micrometer. Cell volume was calculated from cell diameter using the method of Goldrick (12).

Because only two groups were involved in the experimental design, a professional statistician advised that Student's t test would be appropriate for determining significance. Differences between groups at the $P < 0.05$ level were considered to be statistically significant (13).

Results

While initially of a similar body weight, a calorically restricting diet resulted in a significant reduction in body weight, as an actual decrease of over 40% occurred over the 15-week test period, or 2.5%/week (Table I). Body size, as estimated by body length, was similar between groups. Table I also indicates that mean arterial blood pressure was similar between groups, while heart rate was significantly decreased during the transition from the obese to lean condition. Table II contains data relevant to obesity and cardiac size. Total heart weight was significantly decreased with weight reduction. Left ventricular weight was decreased as well. Atrial weight was similar between groups, as was left ventricular thickness. Assessment of heart weight/unit

of body weight (mg/g) indicated that a significant increase in this parameter occurred following weight loss by obese rats. Taken together, these data indicate that the transition from the obese to lean condition primarily involves adaptations in the mass of the left ventricle, but not the thickness, implying an eccentric adaptive pattern. Regardless, the heart remained in a state of adaptive hypertrophy following weight reduction, as illustrated by heart to body weight ratios.

Table III indicates that, upon weight reduction, significant changes in adipose tissue morphology occurred. In Table IV, a variety of renal physiologic values are shown. Weight-reduced rats consumed less water than obese animals, yet excreted approximately 65% of the water consumed, as opposed to approximately 47% in the obese group. Weight-reduced rats were also excreting approximately twice the amount of sodium consumed, whereas sodium balance remained constant in the obese group. An inverse relationship was observed between circulating atrial natriuretic peptide and plasma renin activity. Hematocrit was not affected by the transition from the obese to lean condition.

Discussion

Currently, there is a considerable body of evidence concerning the role of atrial natriuretic peptide (ANP) in acute blood pressure and fluid volume regulation. Importantly, however, the role of ANP in long-term homeostasis of these physiologic variables is not well documented. Obesity is a condition in which extracellular volume expansion occurs slowly, yet consistently, and over extended time periods. In a similar manner, treatment of obesity involving professionally supervised weight loss may occur over months, or even years, with a concomitant reduction and redistribution of fluid volume. Although the transition to and from the obese condition must involve significant adaptations in total blood volume and its redistribution, any role of ANP in this event has not been described.

In obese humans subjected to weight reduction programs, a significant proportion of the decrease in body weight is contributed to by fluid loss (3, 14). This weight loss is achieved in large part through increased diuresis and a concomitant significant excretion of sodium. Interestingly, blood pressure often remains normotensive, but may decrease slightly during weight reduction (15). Although ANP levels have not been recorded in these cases, our experimental data imply that this peptide hormone may play a modulating role in the pathophysiology of obesity. This statement is supported by data indicating that obese rats, after undergoing a considerable reduction in body weight and adipose tissue mass, exhibited elevated circulating ANP, a negative sodium balance, and a normotensive blood pressure. The significant natriuresis required during the transition from the obese to lean state may therefore have been facilitated by ANP.

With respect to the synthetic capability of ANP by obese individuals, some mention should be made of cardiac morphology. Hearts of obese rats were enlarged through volume overload, as exemplified by increased heart weight and left ventricular mass. Atrial dimensions increase in obese humans (16) and spontaneously obese rats, but increased left ventricular mass represents the major cardiac morphologic adaptation to obesity (17). This obesity-induced left ventricular hypertrophy is eccentric, as opposed to the concentric hypertrophy of hypertension (18, 19). Because cardiac hypertrophy

Table I. Morphologic and Hemodynamic Aspects of Experimental Obesity[a]

Group	Initial body weight (g)	Final body weight (g)	Body length (cm)	Mean arterial blood pressure (mm Hg)	Heart rate (beats/min)
Obese	768	721	50.6	113	396
	(21)	(23)	(0.60)	(2.3)	(11)
Weight-reduced	799	472**	49.5	119	358*
	(21)	(12)	(0.82)	(1.6)	(13)

[a] Values are the mean of $n = 7$ rats/group with SEM in parentheses. Significantly different from obese value at * $P < 0.05$ and ** $P < 0.01$.

Table II. Effects of Weight Reduction in the Obese State upon Cardiac Size[a]

Group	Total heart weight (mg)	Atrial weight (mg)	Left ventricular weight (mg)	Left ventricular thickness (mm)	Heart weight/ body weight (mg/g)
Obese	1790	148	1280	4.00	2.50
	(50)	(8)	(46)	(0.12)	(0.067)
Weight-reduced	1400*	121	979*	3.67	2.97*
	(46)	(12)	(38)	(0.20)	(0.067)

[a] Values are mean of $n = 7$ rats/group with SEM in parentheses. All values represent wet weights. Significantly different from obese value at * $P < 0.01$.

Table III. Food Intake, Body Fat, and Adipocyte Morphology in Spontaneously Obese and Formerly Obese, Weight-Reduced Rats[a]

Group	Food intake (g/24 hr)	Adipose tissue weight (g)	Adipocyte volume (pl)	Adipocyte diameter (μm)
Obese	26.6	15.7	626	98.4
	(1.30)	(1.4)	(60)	(6.33)
Weight-reduced	8.7*	2.7*	123*	52.7*
	(0.21)	(0.55)	(15)	(2.77)

[a] Values are mean with SEM in parentheses of $n = 7$ rats/group. Adipose tissue values represent those obtained from the epididymal depot. All weight-reduced values are significantly different from the obese values at * $P < 0.001$.

Table IV. Renal Physiologic Values in Obese and Formerly Obese, Weight-Reduced Rats[a]

Group	Water intake (ml/24 hr)	Urine output (ml/24 hr)	Sodium intake (mg/24 hr)	Sodium excretion (mg/24 hr)	Rat ANP (pg/ml)	Plasma renin activity (ng/ml/hr)	Hematocrit (%)
Obese	56.6	26.6	79.8	77.7	107	5.4	36.6
	(3.31)	(1.65)	(5.66)	(2.01)	(18.3)	(1.2)	(1.19)
Weight-reduced	25.6**	16.8**	26.1**	49.7**	185*	2.0***	35.3
	(0.98)	(0.93)	(0.76)	(3.05)	(13.6)	(0.29)	(1.12)

[a] Values are mean with SEM in parentheses. Significantly different from obese values at * $P < 0.01$, ** $P < 0.001$, and *** $P < 0.05$.

is documented to result in elevated cellular concentrations of atriopeptin (20), the obese heart should have the capability of synthesizing an increased amount of the prohormone. Hypertrophy remained following weight loss, which may have contributed to the elevated circulating ANP observed in formerly obese animals after normalization of body fat. Future experiments assessing concentration of atrial peptides in ventricular tissue from obese animals could provide additional insight into the cardiac adaptive response to this slowly developing, albeit significant, volume-induce hypertrophy.

Importantly, even though obese rats exhibited approximately one half of the circulating concentration of ANP as their weight-reduced counterparts, previous studies from our laboratory have indicated that the cardiac output of these animals is at least 2-fold that of the lean, weight-reduced rats (10). Since intravascular volume correlated directly with cardiac output in hemodynamic studies of obese patients (21), the concentration of ANP assessed per milliliter of circulating plasma may not adequately express the true quantity of hormone released by the obese heart in the face of elevated blood volume. Conversely, the significant elevation of ANP observed in weight-reduced, formerly obese animals may reflect, in part, the reduction in blood volume and not necessarily an increased release of the hormone. Regardless of the synthetic capability of the heart, our studies indicated that obese rats excrete considerable quantities of sodium during weight reduction, which occurred along with elevated concentrations of circulating ANP and a reduced plasma renin. Future studies assessing similar endocrine responses in lean animals prevented from become obese could provide additional information concerning the mechanistic basis of the observed change in ANP concentration. Currently, however, our study has provided new information on the role of this peptide hormone as a modulating factor during chronic body fluid expansion, as opposed to its already documented effects in acute models of cardiovascular trauma (22, 23).

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