Biphasic changes in heart performance with food restriction in rats

KELLY A. McKNIGHT, HEINZ RUPP, KEN S. DHALLA, ROBERT E. BEAMISH, AND NARANJAN S. DHALLA
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McKnight, Kelly A., Heinz Rupp, Ken S. Dhalla, Robert E. Beamish, and Naranjan S. Dhalla. Biphasic changes in heart performance with food restriction in rats. J. Appl. Physiol. 87(5): 1909–1913, 1999.—To examine effects of food restriction resembling very-low-calorie dieting on heart performance, normal rats were fed 25% of ad libitum food intake for 14 days. Although heart weight decreased (P < 0.05) after 5 days, left ventricular systolic pressure as well as rates of pressure development and fall were increased (P < 0.05) at 7 days and decreased (P < 0.05) after 14 days. Systolic and diastolic blood pressures were also increased from 5 to 7 days and decreased after 14 days. The increased hemodynamic performance of heart was associated with a raised plasma norepinephrine concentration, which peaked at day 7 of food restriction; epinephrine concentration was increased (P < 0.05) also at day 7. An increased catecholamine synthesis was indicated by the raised (P < 0.05) plasma dopamine β-hydroxylase activity at 3 days, but this was decreased (P < 0.05) at 14 days. The concentration of dopamine in the heart was increased (P < 0.05) at 2–14 days, of norepinephrine at 7–14 days, and of epinephrine at 10 and 14 days. Food restriction thus appears initially to be associated with an enhanced catecholamine influence on the heart and is followed by a depressed cardiac performance.

cardiovascular function; low-calorie dieting; sympathetic activity; plasma catecholamines; cardiac catecholamine stores

In westernized societies, eating disorders represent a major factor contributing to the high cardiovascular morbidity and mortality. Of particular relevance are overweight-associated diseases such as hypertension, coronary heart disease, and diabetes mellitus (5). Despite the increasing evidence that cardiovascular diseases are aggravated by overweight, weight loss programs did not emerge as straightforward strategies for normalizing body weight (10). One of the reasons is that neuroendocrine events during weight reduction remain poorly understood. Particularly ill defined are processes associated with very-low-calorie dieting involving a greatly reduced food intake (21). This type of dieting appears, however, to be common in North America and is typically followed by an excess calorie intake resulting in weight cycling. About 20% of the population have been estimated to be on weight-lowering diets (6). Furthermore, individuals often start weight-lowering diets even though they are not overweight (4, 12). Previous studies in humans and animals showed a depression in cardiovascular function after a reduced food intake (1, 3, 15, 19). Fluctuations in plasma and tissue catecholamines as a consequence of alterations in biosynthesis, release/uptake, and metabolism may account for the depressed cardiovascular parameters (7, 8, 14, 29). We have shown previously that a prolonged food restriction for 14 days results in bradycardia, hypotension, and decreased rates of cardiac contraction and relaxation (22); plasma norepinephrine levels were, however, elevated (22). This intriguing finding was attributed to downregulated β-adrenergic receptors in the heart (22). In accordance with these findings, epinephrine neither increased contractile force development nor induced arrhythmias (22). A reduction in plasma norepinephrine concentration was observed only after 28 days of food restriction (17). It remained, however, undefined when the depression in heart performance occurred and whether short-term food deprivation had opposite effects. We examined, therefore, the hypothesis that food deprivation is initially associated with a rise in plasma catecholamines followed by depressed values. It was also hypothesized that initial signs of adrenergically mediated stress due to food deprivation are associated with an altered performance of the heart.

We carried out a time course study focusing on cardiovascular parameters after imposing a greatly reduced calorie intake in normal-weight rats. As a model of very-low-calorie dieting, we reduced food intake to 25% of ad libitum intake. After 1, 2, 3, 5, 7, 10, and 14 days, markers of catecholamine influences on heart muscle were assessed. The present study demonstrates that food restriction of normal-weight animals results in a biphasic change in heart performance. A biphasic change was also observed in plasma dopamine β-hydroxylase activity, which was increased 3 days after food restriction and reduced after 14 days. The plasma norepinephrine concentration peaked at 7 days but remained elevated throughout the period of food restriction. Thus food restriction of normal-weight rats appears initially associated with adrenergically stimulated stress due to food deprivation and is associated with an altered performance of the heart.

METHODS

Feeding schedule. Male Sprague-Dawley rats weighing 350–400 g were acclimated for 1 wk and randomly divided into two groups for a dietary regime of control, i.e., ad libitum fed and fed 8 g/day of regular rat chow. A preliminary study...
determined that this amount of chow corresponded to 25% of ad libitum food intake. Water was provided ad libitum. All rats were housed in individual cages in a 22°C room with a 12:12-h light-dark cycle. After 1, 2, 3, 5, 7, 10, and 14 days of dietary restriction, the following measurements were performed.

Heart performance. Rats were anesthetized with pentobarbital sodium (50 mg/kg ip). Electrocardiograph (ECG) electrodes were applied for a lead II configuration. The trachea was intubated to ensure an open airway. The right carotid artery was isolated, separated from the vagus nerve, and ligated with a 5-0 silk tie at the proximal end. A bulldog clamp was applied to the artery ~3 cm distal to the ligation site. A micropipette pressure transducer (model SPR-249, Millar Instruments, Houston, TX) was introduced through a butterfly intravenous needle was inserted into the right carotid artery, the bulldog clamp from the right carotid artery was removed, and blood was collected into an ice-cold heparinized vacutainer tube. The blood was centrifuged at 3,000 × g for 10 min, and the plasma was separated and frozen at -70°C.

Catecholamines and dopamine \( \beta \)-hydroxylase. A no. 23 butterfly intravenous needle was inserted into the right carotid artery, the bulldog clamp from the right carotid artery was removed, and blood was collected into an ice-cold heparinized vacutainer tube. The blood was centrifuged at 3,000 × g for 10 min, and the plasma was separated and frozen at -70°C. The heart, kidney, and cortex were excised and submerged in cold saline. The atria and connective tissue were trimmed away, and the ventricles were weighed. Each tissue was homogenized with 0.4 N perchloric acid, and the homogenate was centrifuged at 18,000 × g for 10 min. The supernatant was carefully aspirated and frozen at -70°C. Catecholamines in tissues and plasma supernatant were extracted with activated aluminum oxide and measured by high-performance liquid chromatography (18). Freezing of plasma or supernatant had no significant effect on catecholamine levels (data not shown). With use of a radioenzymatic assay, dopamine \( \beta \)-hydroxylase activity was assayed in the plasma (16).

Statistical analysis. Results are expressed as means ± SE. Statistical comparisons were made by unpaired two-tailed Student’s t-test by using the Bonferroni correction. Statistical significance was assumed at \( P < 0.05 \).

RESULTS

Rats fed 8 g regular chow/day for 1–14 days exhibited a progressive reduction in general growth characteristics (Table 1). From 5 days onward, both body and heart weight were decreased (\( P < 0.05 \)) compared with ad libitum-fed rats. Despite the reduced heart weight, the food-restricted rats exhibited increased systolic, diastolic, and mean blood pressures from day 5 to day 7 (Table 1). Only at 14 days after food restriction, blood pressure was lower (\( P < 0.05 \)) than the starting value. To assess whether the increased blood pressure was associated with an enhanced heart performance, we monitored hemodynamic parameters of the left ventricle. Systolic pressure development was increased (\( P < 0.05 \)) from 2 to 7 days and was decreased at 14 days (Fig. 1). Left ventricular end-diastolic pressure was increased from 3 to 7 days but was not reduced below starting value at 14 days (Fig. 1). \(+ dP/dt\) and \(- dP/dt\) were increased (\( P < 0.05 \)) from 2 to 7 days and decreased at 14 days of food restriction (Fig. 2). Heart rate was not significantly altered during the first 7 days of food restriction but was decreased (\( P < 0.05 \)) at 10 and 14 days after food restriction (Fig. 2).

To assess whether the enhanced heart performance observed after the initiation of food restriction could be

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<th>Table 1. Time course of growth parameters and blood pressure in rats fed a restricted diet</th>
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Values are means ± SE of 6 rats; values for control groups represent 21 rats. AoSP, aortic systolic pressure; AoDP, aortic diastolic pressure; MAP, mean arterial pressure. *Significantly different from the respective control value, \( P < 0.05 \).
attributed to increased catecholamine influences, catecholamines were determined in the plasma taken from animals after the hemodynamic measurements were performed. Plasma norepinephrine concentration increased (\(P < 0.05\)) from day 1 to day 14 and peaked on day 7 of food restriction (Fig. 3). Although plasma epinephrine concentration showed increases in food restricted rats, it was significantly elevated at day 7 only. Plasma dopamine concentrations did not change significantly (Fig. 3). The activity of dopamine \(\beta\)-hydroxylase in the plasma was increased (\(P < 0.05\)) on day 3 and decreased (\(P < 0.05\)) on day 14 of food restriction (Fig. 4). In contrast to heart performance, plasma norepinephrine concentration and dopamine \(\beta\)-hydroxylase activity, no biphasic changes were observed in cardiac catecholamine concentrations.

Norepinephrine concentrations in heart muscle were increased (\(P < 0.05\)) at 7–14 days, whereas heart epinephrine concentrations were increased (\(P < 0.05\)) at 10 and 14 days (Fig. 5). Dopamine concentration rose (\(P < 0.05\)) above the control level at 2–14 days.
examine whether the changes observed in cardiac catecholamine concentrations were organ specific, catecholamine concentrations were determined also in the kidney and the cortex. The kidney norepinephrine concentration was increased (P < 0.05) only at 14 days after food restriction (Table 2). No significant changes in norepinephrine, epinephrine, or dopamine were observed in the cortex (Table 2).

DISCUSSION

The aim of the present study was to gain insight into the time course of cardiac dysfunction during a greatly reduced calorie intake of normal-weight animals. Calorie intake of rats was adjusted to 25% of ad libitum intake, resembling very-low-calorie dieting, which represents a common practice of dieting for weight reduction. Although the present study was performed with the normal-weight animals, it appears that the observed depressed heart performance and sinus bradycardia are common features of a prolonged markedly reduced food intake; this has been observed also in obese persons after very-low-calorie dieting (30, 31). A decreased muscle sympathetic nerve activity has also been reported after a body weight reduction (2). Urinary catecholamine excretion was, however, found unchanged after 8 days of very-low-calorie dieting (23). A novel observation of the present study is the biphasic change in cardiovascular parameters and plasma catecholamines after the restriction of food intake of normal-weight animals.

Plasma dopamine β-hydroxylase activity was increased at 3 days of food restriction and decreased after 14 days. Also plasma concentrations of norepinephrine and epinephrine were increased initially. Because plasma catecholamines are influenced by central, peripheral sympathetic and sympathoadrenal sources as well as by uptake, turnover, degradation, and binding to postsynaptic receptors (13), no conclusion on the source of the increased catecholamine concentrations can be drawn. In this respect, it should be mentioned that an exaggerated norepinephrine turnover rate and higher plasma epinephrine level have been observed in fasted rats subjected to exercise (20). Furthermore, the psychological stress due to a novel environment resulted in a greater norepinephrine release in fasted rats (9). This suggests that during food restriction the body has a lower threshold for triggering catecholamine release.

Systolic pressure development was increased during 2 to 7 days but decreased at 14 days of food restriction. Furthermore, left ventricular end-diastolic pressure was increased during 3–7 days, whereas heart rate was depressed only at 14 days. Although plasma catecholamine levels are considered to be one of the determinants of cardiovascular activity and can be seen to explain the augmented systolic pressure development during 2–7 days of food restriction, a depressed cardiovascular performance at 14 days was seen in the presence of slightly elevated levels of circulating catecholamine. The depressed performance at 14 days could, however, be attributed to previous observations of a greatly reduced adrenergic responsiveness of the heart (21, 22). Because possible changes in peripheral resistance and hydration status or intravascular volume were not monitored, the hemodynamic measurements cannot be interpreted in terms of possibly altered myocardial performance. It should, however, be noted that in fasted rats biochemical changes, such as a reduced Ca2+-stimulated ATPase activity of the sarcoplasmic reticulum Ca2+-pump and reduced myosin isozyme V1 proportion, have been observed (26), and these can be seen to contribute to the altered cardiac performance.

The present data demonstrate that changes in tissue catecholamine concentration not only depended on the duration of food restriction but were also organ specific. Thus cardiac norepinephrine concentration increased significantly during 7–14 days, epinephrine concentration increased during 10–14 days, and dopamine concentration increased during 2–14 days. Although a decrease in heart weight occurred in diet-restricted animals, it cannot account for the increased catecholamine concentrations because the magnitudes of these changes varied differently. The pattern of these changes is specific for the heart because brain catecholamines were not affected and kidney norepinephrine concentration was increased at 14 days only. Because heart muscle exhibits only a low activity of phenylethanolamine N-methyltransferase (27), the increased level of epinephrine in the heart from dietary-restricted animals arises most probably from uptake from the circulation. The increased levels of norepinephrine are indicative of an enhanced biosynthesis (24).

In conclusion, food restriction of the normal-weight rats has a biphasic action, whereby initially cardiovascular parameters as well as plasma dopamine β-hydroxylase activity are increased. After a prolonged period of food restriction, heart performance was, however, depressed. The initial increase in plasma catecholamines is attributed to the stress of food deprivation. After a prolonged period of fasting, depressed plasma norepinephrine concentrations were, however, observed, which are characteristic for a chronically reduced calorie intake (17). Because high levels of catecholamines can have additional deleterious effects giving rise to an “excess catecholamine syndrome” (11, 25), enhanced catecholamine influences shortly after

| Table 2. Tissue catecholamines in rats fed a restricted diet |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Cortex          | Kidney          |                |                |
|                | NE   DA         | NE   DA         |                |                |
| Control        | 239 ± 57        | 1,196 ± 190     | 107 ± 18       | 13 ± 3         |
| 1 day          | 289 ± 67        | 1,238 ± 237     | 120 ± 20       | 8 ± 1          |
| 2 days         | 386 ± 58        | 1,755 ± 313     | 214 ± 130      | 11 ± 2         |
| 7 days         | 325 ± 43        | 1,496 ± 183     | 158 ± 29       | 21 ± 2         |
| 14 days        | 284 ± 36        | 1,430 ± 165     | 284 ± 32*      | 26 ± 5         |

Values are means ± SE of 6 rats for each group given in ng/g. NE, norepinephrine; DA, dopamine. *Significantly different from respective control value, P < 0.05.
food restriction appear to have unfavorable consequences. In view of the high incidence of intermittent low-calorie dieting, particularly in persons who are borderline overweight, the action of recently developed drugs such as imidazoline agonists that reduce sympathetic outflow of the brain (25, 28) deserves to be investigated.

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