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Serial Changes in Cardiovascular and Renal Function of Rabbits Ingesting a High-Fat, High-Calorie Diet

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To explore the mechanisms of obesity-induced hypertension we analyzed the sequential changes in cardiovascular and renal function in adult rabbits switched to high-fat diet (HFD) for 8 weeks. Animals were housed in metabolic cages for continuous 24-h recording of arterial pressure by telemetry and daily urine collection. High-fat diet induced a progressive increase in body weight (+47%) and a rapid rise in mean arterial pressure, heart rate, and glomerular filtration rate that stabilized, respectively, at 14%, 31% and 68% greater than control values. Time-course analysis of changes in blood pressure may reveal two components of obesity-induced hypertension, an early phase related to HFD itself and a later phase related to weight gain. Am J Hypertens 1999; 12:826–829 © 1999 American Journal of Hypertension, Ltd.

KEY WORDS: **Obesity, hypertension, rabbits, high-fat diet, telemetry.**

Numerous epidemiologic and clinical studies have reported a strong correlation between body weight and blood pressure.[1,2] However, these studies are mainly correlative in nature, and the basic mechanisms relating obesity to hypertension are not clear. To elucidate these mechanisms, many experimental models of obesity have been developed. In most models the obesity is already established by the time of adult age (genetic models) or occurs in small animals (mice and rats) in which extensive sequential endocrine and cardiovascular studies are not feasible. Models with dogs fed a high-fat diet have been used successfully by us[3] and others.[4,5] However, the dog model is expensive, extremely labor-intensive, and may require force-feeding to obtain a suitable weight gain.

An interesting alternate model developed recently[6] uses adult rabbits fed an ad libitum high-fat diet (HFD). These rabbits become spontaneously obese and exhibit hemodynamic and neurohumoral changes that mimic changes observed in human obesity. In this early study,[6] the rabbits were studied only after weight gain had occurred. Yet, sequential humoral and hemodynamic measurements are often helpful to determine the mechanisms of certain forms of hypertension, as early changes of relevant parameters may help establish a causal relationship and reveal the logical sequence of events. To further characterize this model, we studied freely moving rabbits during the development of obesity, with special emphasis on monitoring renal function and blood pressure by telemetry 24 h a day.

MATERIALS AND METHODS

Animal Preparation Adult male lop-eared rabbits (26.3 ± 1.6 week old; body weight, 4.04 ± 0.11 kg) of

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the breed "Bélier Français" were used in this study. All protocols were approved by the State Animal Committee. Using halothane anesthesia and aseptic techniques, the rabbits were instrumented with an arterial catheter connected to an implantable transducer (model TA11PA-C40, Data Sciences International, St. Paul, MN) and radio transmitter to monitor arterial pressure by telemetry. Through a groin incision, the arterial pressure cannula was inserted into the femoral artery, and the body of the implant was attached to the abdominal wall. After surgery, the rabbits were housed in individual cages, customized for urine collection, in a quiet air-conditioned room with a 12-h light-dark cycle. The rabbits were allowed to recover for at least 10 days before the start of the experiments. All animals had free access to tap water and were fed 150 g of standard rabbit chow per day.

Continuous Hemodynamic Monitoring by Telemetry Each cage (65 cm × 70 cm × 60 cm, W×D×H) was equipped with three RLA-2000 receivers, connected through a multiplexer (RMX-10) to a calibrated pressure analog adapter (R11CPA), allowing continuous recording of the analog pulsatile arterial pressure signal. Corrections for changes in barometric pressure were done through the APR-1 pressure reference device. All devices are manufactured by Data Sciences International, St. Paul, MN. The analog pressure signal was then sent to an A/D converter (CIO-DAS08, ComputerBoards, Mansfield, MA) and processed by a personal computer using customized algorithms for beat-to-beat analysis.[7] The signal was sampled at 500 Hz for 5-s periods every 30 s, from 10:00 AM to 8:00 AM the next morning.

Experimental Protocol After a first week of control measurements with standard rabbit chow (150 g/day), the rabbits (n = 8) were fed ad libitum a high-fat diet (standard rabbit chow with 10% added fat as soya oil: pork fat, 1:1) for 8 weeks. Another four rabbits were fed the standard rabbit chow and served as time-controls. Water and food intake, and 24-h urine output, were determined daily at approximately 9:00 AM. Body weight was measured twice a week. Blood samples were withdrawn weekly by arterial puncture of the central ear artery for various analytic measurements (see below).

Analytical Measurements Sodium and potassium were determined by flame photometry (model IL 943, Instruments Laboratories, Lexington, MA), glucose by the glucose oxidase method (Beckman glucose analyzer 2, Beckman, Fullerton, CA), and creatinine by the kinetics of Jaffé (Beckman creatinine analyzer 2). Glomerular filtration rate (GFR) was assessed based on creatinine clearance.

Statistical Analysis Statistical analysis within group was performed by analysis of variance for repeated measurements and multiple comparison procedures. Because each rabbit within a group serves as its own control, experimental values were compared with control values in each group using Dunnett's multiple comparison procedure. Changes were considered to be statistically significant if $P < .05$.

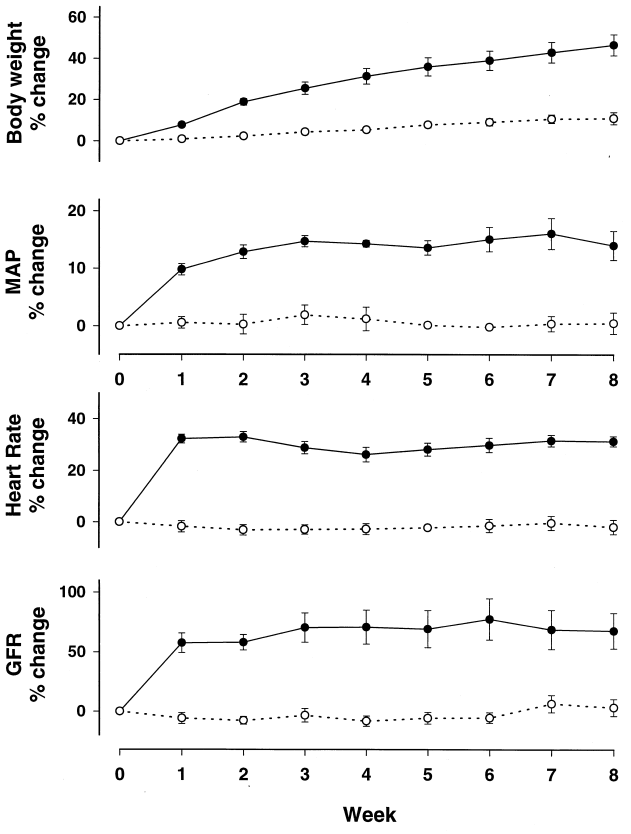

FIGURE 1. *Percent changes in body weight, mean arterial pressure, heart rate, and glomerular filtration rate in rabbits given 8 weeks of high-fat diet (solid line, closed symbols) or in time-control rabbits (dotted line, open symbols). Values are expressed as mean ± SE. All changes in high-fat diet animals, compared with the control week (week 0), were significant ($P < .05$).*

RESULTS

Figure 1 illustrates the percent changes in body weight, mean arterial pressure, heart rate, and glomerular filtration during 8 weeks of high-fat diet. There was a progressive increase in body weight, from 4.05 ± 0.15 kg to 5.91 ± 0.09 kg (+47 ± 5%), (n = 8, $P < .05$). Mean arterial pressure rose rapidly from a control value of 65.4 ± 2.9 mm Hg to 73.8 ± 3.2 mm Hg (+12.9 ± 1.2%) after 2 weeks of HFD (n = 8, $P < .05$). Surprisingly, the increase in MAP was significant already after three days of the high-fat diet (+8.9 ± 1.5%) and attained plateau values by the end of the first week. After eight weeks of HFD, mean arterial pressure was 14.1 ± 2.5% ($P < .05$) above control

values (n reduced to 4 due to implant failure). Heart rate rose significantly from a control value of 164 ± 3 bpm to 217 ± 4 beats/min (+32.2 ± 1.7%, n = 8) during the first week of HFD. This increase was already present at the very first day after diet switch, and remained elevated over the rest of the experiment (+31.3 ± 2.0%, week 8, $P < .05$). Glomerular filtration rate rose significantly from 8.9 ± 0.4 to 13.9 ± 0.8 mL/min (+57 ± 8%, week 1) and, as shown in Figure 1, remained elevated throughout the remaining weeks (+68 ± 15%, week 8, n = 8).

The rabbits showed a significant positive daily sodium balance (sodium intake minus urinary sodium excretion) during the first 3 weeks of HFD, compared with the control week. In the following weeks, daily sodium balance returned gradually toward control levels. This resulted in a net accumulation of sodium over the whole experimental period. Potassium balance data showed a sustained potassium retention. Rabbits fed the high-fat diet tended to show a slight elevation in plasma glucose (+7 ± 2% at weeks 7 to 8, compared with a control value of 7.4 ± 0.2 mmol/L), but this increase was not significant. At the end of 8 weeks of HFD, fat intake was limited for the next 10 days to avoid further weight gain. Mean arterial pressure and heart rate remained elevated (76.4 ± 6.8 mm Hg and 213 ± 7 beats/min, respectively, during the last 7 days of the 10-day period , versus 76.0 ± 6.6 and 216 ± 6 during the eighth week of ad libitum HFD intake; n = 4). As shown in Figure 1, animals fed the control diet throughout the experiment showed a slight but significant increase in body weight, from 4.02 ± 0.19 kg to 4.46 ± 0.14 (+11.3 ± 2.9%), and no changes in any of the other parameters. There were no implant failures in the control group.

DISCUSSION

Obesity is one of the most serious health problems in industrialized societies, with a rapidly increasing prevalence in the United States[8] and in Europe.[9] The association between obesity and hypertension has been recognized for many decades[10] and epidemiologic studies show a good correlation between body weight and blood pressure both in normotensive and in hypertensive individuals.[1,2] Numerous mechanisms have been postulated to explain obesity-induced hypertension. However, much of our knowledge is based on correlations between body weight and various factors thought to increase blood pressure (such as insulin levels, plasma renin, or sympathetic activity), and the basic physiologic mechanisms that link body weight and blood pressure are not yet fully elucidated.

The present study is consistent with previous reports in rabbits[6,11] that demonstrate that female New Zealand White rabbits fed an ad libitum high-fat diet become obese and develop mild hypertension and tachycardia. In our strain of male rabbits, 8 weeks of HFD induced a 47% increase in body weight, a 14% increase in mean arterial pressure, and a 31% increase in heart rate. Obese rabbits also exhibited changes in renal function, with increased glomerular filtration rate and a net accumulation of sodium. These findings are consistent with observations in the dog model of obesity-induced hypertension.[5] These changes may be due to an increased tubular sodium reabsorption at a site before macula densa (by sympathetic activation, hyperinsulinemia, or renal tubular compression), resulting in a compensatory increase in GFR, as previously hypothesized.[5] These mechanisms could also explain the increased plasma renin activity in obese dogs[5] and rabbits,[6] despite volume retention and increased blood pressure.

A major finding of our study is that the high-fat diet induced a rapid increase in daily mean arterial pressure and heart rate, which occurred in the very first days after a switch from the control to the high-fat diet. Clearly, these early changes cannot be attributed to weight gain. More likely, metabolic changes induced by the high-fat diet and/or overfeeding, such as hyperinsulinemia and sympathetic nervous system activation, may mediate the initial hemodynamic response to the high-fat diet.[12] Impaired flow-mediated vasodilation could also play a role, as it has been reported to occur after a single high-fat meal.[13] In fact, blood pressure returns rapidly to control values when rabbits are switched back to maintenance diet after a single week of HFD (pilot studies, data not shown).

On the other hand, the long-term increase in blood pressure seems to be related more to increased fat mass than to fat intake per se. Indeed, when fat intake was substantially reduced in obese rabbits after 8 weeks of HFD to avoid any further weight gain, blood pressure and heart rate remained elevated. An interesting observation is that the moderate but significant increase (+11%) in body weight of time-control rabbits was not associated with an increase in blood pressure. Based only on gross examination at the autopsy, which revealed massive visceral fat accumulation in HFD rabbits as compared with control animals, we hypothesize that accumulation of fat rather than lean mass might be necessary for hypertension to develop. Although hyperinsulinemia, elevated plasma renin, or tubular physical factors may all play a role in obesity-induced hypertension, an interesting candidate is leptin, the peptide product of the so-called obese gene. Leptin production correlates positively with body mass index[14] and chronic leptin infusion increases both blood pressure and heart rate in rats,[15] probably through sympathetic activation.[16]

In summary, a time-course analysis of the changes in blood pressure during a high-fat diet in rabbits may

reveal two components of obesity-induced hypertension, an early phase that could be due to high-fat intake and/or overfeeding, and a later phase that seems to be strongly related to weight gain and increased fat mass. This rabbit model of obesity-induced hypertension is an attractive and promising system for further studies to elucidate the mechanisms that link weight gain and blood pressure.

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