Effects of Chronic Combined Treatment With Captopril and Pravastatin on the Progression of Insulin Resistance and Cardiovascular Alterations in an Experimental Model of Obesity in Dogs

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Obesity is a metabolic disorder in which multiple clinical and biochemical alterations coexist. However, the progression of these alterations in relation to weight gain has not been investigated in detail. Therefore, we studied the evolution of insulin resistance and associated risk factors in a model of experimental obesity in dogs. We also studied whether chronic exposure to these pathogenic factors could induce cardiac and vascular alterations. Twenty male age- and body weight-matched beagle dogs were divided into four groups (n = 5), according to diet and pharmacologic therapy received, and followed for 2 years. Control animals were maintained with a regular diet, while the 15 remaining animals were fed a high-fat diet. The Obese group of dogs received no therapy, whereas the Capto group received 25 mg/12 h captopril, and the Prava+Capto was treated with 10 mg/24 h pravastatin plus the same dose of captopril throughout the study. Periodical determinations of clinical and biochemical parameters were made, and the degree of insulin resistance was also estimated. After the 2-year follow-up, the dogs were killed and vascular thickening in the aorta and the coronary arteries was evaluated. In addition, cardiac hypertrophy was estimated by heart weight and free-wall left ventricular width. Chronic pravastatin plus captopril treatment, together with decreasing weight gain rate, ameliorated the progression of insulin resistance and associated risk factors (hyperinsulinemia, hypercholesterolemia) related to this severe model. In addition, this combined therapy showed cardioprotective action, as cardiac and vascular hypertrophy observed in the Obese group was prevented. These positive results seems to emerge from the synergistic effects of both drugs, as captopril as monotherapy induced only a slight benefit on these parameters. Am J Hypertens 1998;11:844–851 © 1998 American Journal of Hypertension, Ltd.

KEY WORDS: Obesity, insulin resistance, captopril, pravastatin, vascular hypertrophy, beagle dogs.

Received August 8, 1997. Accepted March 3, 1998.

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This work was supported by Research Project Grant number 93/1, (BMS), from Plan Nacional de Fomento a la Investigación, Spain.

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The concept of obesity as a metabolic disorder has gained acceptance during recent years due to the demonstration of clustered biochemical and hormonal alterations in this syndrome. In this respect, abnormal carbohydrate and lipid profiles are features that are usually present in the obese. Nowadays, it is accepted that the existence of insulin resistance (IR) in these individuals is a dysfunction that underlies all of these alterations. Although some controversy still remains about the intimate mechanism responsible for this metabolic derangement, it has recently been pointed out that the endothelium of the skeletal muscle vasculature is the main target for the deleterious actions leading to impaired insulin-mediated vasodilation in obese humans. However, a defective insulin-dependent vasodilation in the coronary artery has been recently reported in dogs, in which obesity was induced through the same model used by us. In addition, cumulative evidence has shown that elevations in triglycerides, total cholesterol, and low-density lipoprotein (LDL) cholesterol levels, and reductions in high-density lipoprotein (HDL) cholesterol concentrations, which frequently co-cluster in the obese, are associated with increases in atherosclerotic cardiovascular disease and coronary heart disease (CHD). All of these data suggest that the skeletal muscle vasculature is not the only vascular bed injured by weight gain-related effects.

Together with these metabolic alterations, obesity and arterial hypertension frequently coexist in the general population, thus constituting an additional risk factor for target end-organ damage. This high incidence of hypertension usually requires the use of pharmacologic therapy in the obese. However, diverse studies have shown that there is a nonhomogeneous pattern of action on the carbohydrate and lipid metabolisms within the wide range of antihypertensive drugs.

Experimental obesity induced by a high-fat diet in dogs is a model that shares many characteristics with the human syndrome. Therefore, we decided to study the long-term effects of obesity not only on the evolution of insulin resistance and associated risk factors, but also to test the possibility that these biochemical and metabolic alterations are perpetuated through structural changes in the cardiovascular system. Furthermore, we have evaluated the influence of antihypertensive monotherapy with captopril, or in association with cholesterol-lowering pravastatin treatment, on the progression of these alterations.

MATERIALS AND METHODS

Animals For the present study, 20 male beagle dogs were used. The dogs, with an initial body weight of 13.4 ± 1.2 kg, were randomly divided into four groups (n = 5) matched for age and diverse clinical, biochemical, and metabolic parameters (see Basal in Table 1). The control group was maintained throughout the study with a normal diet, whereas the 15 remaining animals were kept on a high-fat diet consisting of beef and lard in addition to the regular food, as previously reported. The Obese group received no pharmacologic therapy during the study, whereas the Capto group was treated with 25 mg/12 h of oral captopril, and the Prava + Capto group received 10 mg/kg/day of pravastatin, a HMG-CoA reductase inhibitor, in addition to captopril at the same dose. The four groups were followed for 2 years with periodic determinations of the diverse parameters.

Periodic Determinations At Baseline and every 3 months after starting the diet or pharmacologic therapy, the dogs were weighed, and fasting values of plasma glucose (PG), insulin (PI), triglycerides (TG), and total cholesterol (tChol) were measured in blood samples. Mean arterial pressure (MAP) was measured in the awake animals by the tail-cuff method with a Dymax 845 device (Critikon Inc., Tampa, FL) validated by our laboratory in a previous study. In addition and at the same periodicity, the degree of insulin resistance (IR) was estimated in every dog by performing an insulin suppression test.

Insulin Suppression Test (IST) After restraining the awake animals, a modification of the IST technique was accomplished by infusing the following solutions through the cephalic vein for 3 h: 1.5–9 mg/kg/min glucose, 1 mU/kg/min insulin, and 0.05 µg/kg/min somatostatin (SS). The SS was used to inhibit endogenous insulin release, therefore allowing the desired level of insulinemia to be reached with the exogenous insulin. The IST was designed to obtain, after 120 min, a steady state of plasma glucose (SSPG), together with a steady state of plasma insulin (SSPI), within the physiologic hyperinsulinemic range. They were both maintained throughout the test. Under these conditions, the insulin sensitivity index (ISI) was calculated as follows: ISI (dl/kg/min) = glucose infusion rate (mg/kg/min) × 10³/SSPG (mg/dL). A lower ISI indicates a higher resistance to insulin-mediated glucose uptake, and vice versa.

Cardiac Measurements and Vascular Histologic Studies At the end of the 2-year follow-up period, each animal was killed, and the heart was removed and rinsed in saline solution. Extracardiac material was discarded before the heart was weighed. Free-wall left ventricular width (LVW) was measured in all the animals as an index of left ventricular hypertrophy (LVH). In addition, the aorta and the coronary arteries were dissected, and arterial rings of both vessels were immersed immediately in formaldehyde. Arterial
Specifications were obtained from several levels along the thoracic and abdominal aorta, and from the origin of the right coronary, anterior descending, and left circumflex coronary arteries. Then, the presence of atheroma plates was sought throughout the length of both arteries in every dog. Arterial samples were sectioned and stained using the hematoxylin-eosin, Masson-trichrome, and oil-red techniques. The media layer width was determined by scoring 10 measurements of the space between the intima and the external laminae in every arterial segment. These blind studies were performed by an independent pathologist.

**Analytical Determinations** Biochemical determinations of plasma TGs and tChol were made by a Hitachi autoanalyzer (model 747), and plasma glucose levels were measured with a glucose analyzer (YSI 23A, Yellow Spring Instrument Co.). Plasma insulin was measured using a commercial radioimmunoassay kit (Sorin Biomedica, Vercelli, Italy).

**Statistical Analysis** Analysis of the data was performed by nonparametric tests, using Freedman’s analysis of variance by ranks to identify global differences between groups, and the Wilcoxon signed rank test for paired comparisons between the different periods of the study. In addition, multiple linear regression models were used to assess the association between the three independent variates (diet, treatment 1, and treatment 2) and the values of diverse clinical, biochemical, and histologic parameters at death. Because of the different weight-gain patterns observed among the groups receiving the high-fat diet, the possibility that body weight might be a confounding factor was also evaluated. It was considered a confounding variable when its removal from the model caused a change in the regression coefficient (RC) of the variable “group” ≥ 10% of the value of this coefficient in the maximal model. A P < .05 was considered significant.

**RESULTS**

**Clinical and Biochemical Parameters (Table 1)** The Control group exhibited no significant modifications in most of the clinical and biochemical parameters under study. However, slight upwards trends were observed in body weight, mean arterial pressure, and fasting plasma glucose and insulin levels along the 2-year follow-up.

On the contrary, the Obese group, in addition to a
significant increase in BW, showed progressive elevations of MAP, PG, PI, tChol, and TG levels, attaining a significant difference compared with both the Basal state and the Control group ($P < .05$ to $.01$) throughout the study.

The Capto group behaved in a manner similar to the Obese group for BW, PG, and tChol, although the increase in these parameters was not as high. MAP values remained comparable to those from the Control group, and fasting hyperinsulinemia showed a slight upwards trend.

Finally, in the Prava+Capto group an intermediate evolution between the Control and the Obese group was observed. The elevation of BW was significant compared with the Basal situation ($P < .04$), although no statistical difference with the Control group was observed ($P = .2$, $P = NS$). No significant change existed for PI and MAP, whereas the rise in tChol was statistically different from both the Control and the Obese groups ($P < .05$).

Captopril in neither monotherapy nor in combination with pravastatin altered the elevation in the level of TG exhibited by the Obese dogs.

**Insulin Resistance Progression (Figure 1)** A significant decrease in the ISI was achieved in the Control dogs at the end of the study, although this change was not observed until the 12-month follow-up. The level before that time was fairly comparable to the Basal measure. From that period, the loss of insulin sensitivity was progressive and statistically significant ($P < .05$ vs Basal).

On the other hand, the insulin sensitivity of the Obese animals was significantly reduced ($P < .05$ to $.001$) when compared with the Basal state; this difference was observed from the 3-month follow-up. This loss of sensitivity was significantly more marked than that observed in the Controls at any time of the study ($P < .05$ to $.01$).

In the Capto group the fall in the ISI level was similar to that of the Obese group, although the slope was smoother until 3 months ($P < .05$ vs Obese). From that follow-up, no significant difference with the Obese group was observed.

Finally, in parallel to most of the clinical and biochemical modifications present in the Prava+Capto group, the degree of the progression of IR in these dogs was also intermediate between those in the Control and Obese groups. Indeed, the ISI was reduced over the course of the study compared with the Basal state, but this decrease showed an intermediate slope between the Control and the Obese groups, which was significantly different from both groups ($P < .05$).

**Cardiac Measurements and Vascular Histology (Table 2)** The high-fat diet in the Obese group was also associated with cardiac hypertrophy, as evidenced by the increased cardiac weight (CW) and free-wall left ventricular thickness when compared with the control animals. In this regard, the dogs maintained with the captopril treatment and high-fat diet showed a significant reduction of both parameters when compared with the Obese group. Chronic combined therapy with pravastatin and captopril completely abolished the cardiac and left ventricular hypertrophies associated with the model, as their values were fairly comparable to those from the Control group. On the other hand, evaluation of the aortic and coronary segments pointed out the existence of a certain degree of arterial hypertrophy associated with this model, as the thickness of the media layer in the Obese group was significantly higher ($P < .01$ to .002) than in the Control animals. Captopril monotherapy prevented vascular thickening in the aortic rings, although only slight positive trends were observed in the coronary arteries.

Finally, pravastatin plus captopril abolished the development of vascular hypertrophy in both the aorta and the three coronary arteries, as evidenced by values of the media layer thickness that were similar to those seen in the Control group.

**Multiple Linear Regression Analysis** Performance of this test showed that body weight was a confounding variable for diverse parameters, such as cholesterol, insulin, ISI, heart weight, and aortic and coronary wall thickness. However, when the analysis was adjusted for this variable the effect of the therapies was still significant for some of the variables. In this respect, the decrease in insulin levels was attributable...
to captopril therapy (RC: -10.96; 95% CI: -16.06 to -5.86). On the contrary and after adjusting for body weight, the cholesterol reduction was demonstrated attributable to pravastatin therapy (RC: -59.3; 95% CI: -91 to -27.6). Furthermore, body weight was a confounding variable for the cardiac and aortic hypertrophy observed in the model, but when the analysis was adjusted for this variable, the effect of both therapies remained significant (P < .05 for HW and aortic thickness with the two drugs). Body weight was also shown to be a confounding variable for coronary hypertrophy (in the three arteries). Adjustment of the analysis for BW revealed that no significant effect was attributable to captopril, whereas pravastatin was responsible for the reduction in the coronary wall thickness of the three arteries (P < .05–.01). Finally, body weight was also a confounding variable for ISI, and when the data were adjusted for BW, diet was found responsible for the decrease in this variable (RC: -49.2; 95% CI: -63.69 to -34.5), whereas, in spite of increases in the mean values, no significant benefit could be attributed to any of the drugs used (RC: 7.2; 95% CI: -7.2 to 21.6 for captopril; and RC: 8.2; CI 95%: -2.9 to 19.3 for pravastatin).

**DISCUSSION**

In the present study we have investigated and characterized the chronic effects of experimental obesity, induced by a high-fat diet, on the derangement of diverse clinical, biochemical, and metabolic parameters. In previous experiments, our group and others have documented the alterations produced by this model on some of these parameters during shorter periods of time. However, to our knowledge, this is the first determination of the changes caused by a high-fat diet after such a long-term follow-up. This prolonged (2-year) period has also allowed us to evaluate the incidence of additional structural (vascular and cardiac) alterations, which were undetectable during shorter studies.

Our findings in the Control group, which showed a certain loss of insulin sensitivity in the second year of the follow-up, might be attributed to age-related effects and the sedentary life maintained by the dogs throughout the study, as none of the evaluated parameters was statistically altered. In this respect, although nonsignificant, the reduction in femoral arterial flow (~13%) and increase in femoral vascular resistance (~30%), both of which could contribute to IR progression, have recently been reported in beagle dogs.9,10

Weight gain in this model was accompanied by the presence of sympathetic overactivity, hyperglycemia, hyperinsulinism, and dyslipidemia (with elevated fasting triglycerides and total cholesterol levels), together with increased blood pressure, as previously reported.9,10 Furthermore, the cardiac and vascular hypertrophy present in our Obese dogs can be considered secondary to their chronic exposure to pathogenic factors associated with weight gain. Herein, we have to accept, as a limitation of the study, that with the present design disclosure between the effects strictly attributable to weight gain and those derived from the type of diet used to overfeed the dogs is not allowed. However, to our minds, because of the close relationship between overweight and fat-enriched diets among the Westernized populations the repercussions of this design deficiency are relevantly minimized. In addition, the relative importance of the type of diet compared to mere weight gain in the pathogenesis of cardiovascular disease emerges also from the different effect of similar increments in BW on insulin resistance between the Control group at the end of the study (~1.5 kg) and the Prava+Capto group at 3-month follow-up. On the other hand, pharmacologic therapy directed to attenuate some of the pathogenic factors associated

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**TABLE 2. HEART WEIGHT, FREE-WALL LEFT VENTRICULAR THICKNESS, AND MEDIA LAYER THICKNESS OF THE AORTIC ARTERY, AND RIGHT, ANTERIOR DESCENDING, AND LEFT CIRCUMFLEX CORONARY ARTERIES AT DEATH**

<table>
<thead>
<tr>
<th></th>
<th>AA  (µm)</th>
<th>RCA (µm)</th>
<th>ADCA (µm)</th>
<th>LCCA (µm)</th>
<th>HW  (g)</th>
<th>FLVT (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (n = 5)</td>
<td>588.9 ± 16.7</td>
<td>124.9 ± 7.7</td>
<td>123.5 ± 6.0</td>
<td>132.1 ± 4.0</td>
<td>110.5 ± 8</td>
<td>0.98* ± 0.05</td>
</tr>
<tr>
<td>Obese group (n = 5)</td>
<td>683.3 ± 22.2</td>
<td>141.5 ± 12.8</td>
<td>153.2 ± 8.0</td>
<td>153.2 ± 19.1</td>
<td>119.5 ± 6</td>
<td>1.22 ± 0.20</td>
</tr>
<tr>
<td>Capto group (n = 5)</td>
<td>639.9 ± 18.4</td>
<td>140.4 ± 7.4</td>
<td>143.8 ± 10.0</td>
<td>146.4 ± 8.0</td>
<td>115.1 ± 9</td>
<td>1.05* ± 0.04</td>
</tr>
<tr>
<td>Prava+Capto group (n = 5)</td>
<td>602.5 ± 11.3</td>
<td>129.5 ± 15.1</td>
<td>130.4 ± 11.4</td>
<td>136.1 ± 9.7</td>
<td>108.9 ± 7</td>
<td>1.04* ± 0.12</td>
</tr>
</tbody>
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Abbreviations: AA, aorta artery; RCA, right coronary artery; ADCA, anterior descending coronary artery; LCCA, left circumflex coronary artery; HW, heart weight; FLVT, free-wall left ventricular thickness.

* P < .01 v Obese group.
† P < .05 v Control and Prava+Capto groups.
‡ P < .002 v Control and Prava+Capto groups.
§ P < .05 v Obese, Control, and Prava+Capto groups.
with the present model partially prevented the metabolic and cardiovascular damage observed in the Obese dogs. In this respect, captopril monotherapy exerted a slightly positive effect on insulin resistance progression, although this difference was only significant in the early phases of the follow-up (3 months). This positive trend was lost in further periods, although the absolute values of the ISI were always higher in the Capto group. At death, evaluation of the heart and arterial samples gave evidence that captopril maintained this slight beneficial trend in the coronary arteries, whereas it partially prevented the thickening of the aortic media layer and cardiac hypertrophy. These latter positive effects were independent of the lower body weight observed in this group during the study compared with the Obese group. To this slight improvement, captopril might have contributed by decreasing blood pressure, fasting insulin, and norepinephrine concentrations, as well as blocking angiotensin II generation, because all these factors are potential promoters of vascular and cardiac remodeling. Indeed, insulin has been reported to stimulate, either directly or by activation of the insulin-like growth factor (IGF-1) receptors, vascular smooth muscle cell and cardiac myocyte hypertrophy. Angiotensin II 17–20 and sympathetic activation, with high norepinephrine concentrations, 21–23 have also been implicated in vascular and cardiac hypertrophy. The hypertrophic properties of these factors probably contributed to the thickening of the left ventricle and the media layer of the aorta and the coronary arteries observed in our Obese dogs. However, reduction of these potential risk factors by captopril was insufficient to prevent the metabolic and end-organ target damage. A possible reason for this lies with the severity of the present model, as evidenced by the approximate 60% increase in body weight, and 80% decrease in insulin sensitivity in the Obese animals at the end of the 2-year follow-up. Prolonged exposure of the animals to this harmful situation can explain the contrast with previous studies using cholesterol-lowering drugs have documented positive effects on cardiovascular morbidity and mortality in individuals with or without previous coronary heart disease. In summary, in this chronic (2-year) model of obesity in dogs, combined therapy with pravastatin plus captopril attenuated the negative clinical, biochemical, metabolic, and cardiovascular effects associated with experimental obesity induced by a high-fat diet in dogs. Although body weight was demonstrated to be a confounding variable for most of the parameters under study, the protective effect observed in the Prava+Capto group is more related to the synergistic actions of both drugs on most of the existing metabolic and cardiovascular risk factors, rather than to mere weight gain restriction.

**ACKNOWLEDGMENTS**

We thank Celia Cuasante and María Eugenia Vera for excellent technical assistance, and Dr. Victor Abraira (Bioclinical Statistics Unit) for his statistical advice.

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