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Effects of Hyperinsulinemia on vascular blood flows in experimental obesity ☆

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Abstract

Human obesity, which is very common in Polycystic Ovaries Syndrome and in "*X* Syndrome", constitutes an insulin-resistance state in which multiple clinical, biochemical and hemodynamic alterations coexist. Insulin resistance in the obese has been recently associated with an endothelial dysfunction. To investigate the possibility that clinical and metabolic derangements related to insulin resistance could induce changes in vascular blood flows, we have studied the levels of mesenteric (MBF), renal (RBF) and femoral (FBF) blood flows in Beagle dogs kept for 2 years on a normal (control group) or high fat diet (obese group). This experimental model exhibits many of the abnormalities with the human syndrome. In addition, we have tested the effects of chronic treatment with captopril (capto group) in monotherapy or in association with pravastatin (prava + capto group) on the hemodynamic changes associated with this diet. After the two year follow-up, Transonic flow probes were placed around the three arteries to measure basal blood flows and their response to a hyperinsulinemic-normoglycemic test in anesthetized animals. During this test the degree of insulin sensitivity was estimated. In association with higher body weight, blood pressure, insulin resistance, and fasting levels of insulin and total cholesterol, the obese group exhibited decreased basal levels of FBF and a greater femoral vasoconstriction during hyperinsulinism ($P < 0.05$ vs control). Combined therapy with captopril and pravastatin ameliorated the reduction in basal FBF and hyperinsulinism-induced vasoconstriction ($P < 0.05$), in addition to the beneficial effects on insulin sensitivity, and clinical and metabolic parameters. Synergistic beneficial effects of both drugs on lipid and carbohydrate profiles may account for this positive outcome, by attenuating the atherogenic process associated with this model.

1. Introduction

In several disorders, as Polycystic Ovaries Syndrome and "*X* Syndrome", it is a common finding that the presence of obesity and insulin resistance, may predispose to coronary heart disease [1]. Obesity can be considered an insulin-resistant state which comprises a constellation of clinical, biochemical and hemodynamic abnormalities. As in other insulin-resistant situations, like aging [2], hypertension [3] or noninsulin-dependent diabetes mellitus (NIDDM) [4,5], obesity is characterized by defective insulin-mediated and endothelium-dependent vasodilation [6,7]. Coexistence of diverse pathogenic circumstances can converge to present this final picture in humans. In this respect, increased blood pressure levels, chronic hyperinsulinemia and an abnormal lipid profile are major risk factors with a high incidence in the obese population [8,9]. These risk factors have the capacity to induce hemodynamic and structural modifications in the vasculature, thereby contributing to the genesis and/or maintenance of insulin resistance [10]. Furthermore, this clustering of arterial hypertension and dyslipidemia in obese subjects usually requires combined pharmacological therapy. However, several studies have pointed out differential effects on the lipid and carbohydrate profiles with diverse antihypertensive therapies [11]. Angiotensin-converting enzyme inhibitors [12] and selective α_1-receptor blockers [13,14] have shown positive effects on insulin sensitivity in hypertensive

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patients. On the other hand, it is also well-known that chronic dyslipidemia can hasten the atherogenic process thus leading to the onset of vascular pathologies of hypertension and atherosclerosis. In a recent publication, chronic use of pravastatin has been reported to induce a positive effect on reducing both cholesterol and insulin levels in elderly hypertensive hypercholesterolemic subjects regardless of the type of antihypertensive therapy used [15]. Furthermore, chronic treatment with pravastatin has provided improvement in endothelium-dependent coronary vasomotion in hypercholesterolemic patients [16]. The mechanisms underlying this protection are not completely understood. However, cumulative data point to the peripheral vasculature as the main target for the deleterious actions associated with insulin resistance, since skeletal muscle is the tissue responsible for glucose disposal. Indeed, decreased peripheral blood flow, secondary to increased sympathetic activity and vessel rarefaction and hypertrophy, has been suggested as one of the steps in the cascade of events leading to insulin resistance [10]. In addition, recent evidence has suggested the possibility that endothelial dysfunction (mainly related with nitric oxide) might account for insulin resistance in the obese [7].

In the present study we have tested the hypothesis that experimental obesity, induced by long-term high fat diet, could trigger chronic hemodynamic alterations in different vascular beds (mesenteric, renal and femoral), in association with metabolic disorders. In addition, we have studied the effectiveness of chronic antihypertensive therapy with captopril, a converting enzyme inhibitor (CEI), alone or in combination with pravastatin, a HGM-CoA reductase inhibitor, in the prevention of possible deleterious blood flow alterations in these vascular territories, related to weight gain.

2. Materials and methods

2.1. Animals

Twenty male Beagle dogs with initial body weight of 13.4 ± 1.2 kg were randomly divided into four groups ($n = 5$) matched for initial values of the following parameters: body weight (BW), and fasting levels of plasma glucose (PG), insulin (PI), total cholesterol (Chol) and triglycerides (TG). The control group was maintained for 2 years on a regular dog pellet chow diet. The remaining fifteen animals were kept throughout the follow-up on a high fat diet consisting of beef fat and lard in addition to their regular diet. During this period, five of these animals received no pharmacological treatment (obese), while five animals received 25 mg/12 h of captopril p.o. (capto), and a fourth group received the same dose of captopril plus 10 mg/day of pravastatin p.o. (prava + capto).

At the end of the 2-year follow-up and after overnight fasting, the conscious animals were weighed, and blood pressure was measured by a tail-cuff method (Dynamap 845, Critikon Inc., Tampa, FL), previously validated by our group [17]. In addition, blood samples were collected for biochemical and hormonal determination.

2.2. Surgical procedure

The animals were anaesthetised with 30 mg/kg of intravenous sodium pentobarbital and, after endotracheal intubation, they were ventilated according to the nomogram of Kleiman and Radford [18]. The right femoral artery was catheterized for continuous blood pressure monitoring and blood sample withdrawal, while the vein was cannulated to infuse additional anaesthesia. A third catheter was inserted into the right cephalic vein for the infusion of the solutions of the insulin suppression test (IST). Through a left flank incision, Transonic flow probes (Transonic System Inc., NY) were placed around the mesenteric and renal arterial segments proximal to the aorta for continuous blood flow monitoring. An additional blood flow probe was set in the contralateral femoral artery to estimate blood supply to the hindlimb skeletal muscle bed.

2.3. Insulin suppression test

After the surgical procedure, saline infusion was started through all the catheters at the same rates used during the test. Then the animals were allowed to stabilize for 45–60 min, and care was taken to observe steady values of the hemodynamic parameters before any further manipulation. At that point, basal levels of the three blood flows and blood pressure were continuously recorded during the 15 min basal period. Thereafter, a modification of the IST technique, described by Shen et al. [19], was performed by intravenous infusion of the following solutions for 3 h: 1 mU/kg/min of insulin, 1.5–9 mg/kg/min of glucose and 0.05 μg/kg/min of somatostatin. Glucose rate was adjusted for each animal to achieve an euglycemic response. Under these conditions, steady state levels of plasma glucose (SSPG) and insulin (SSPI) were reached after 120 min and maintained throughout the test. Arterial blood samples for analytical determinations were drawn every 10 min along the last hour. Along this last hour, continuous recordings of blood pressure and mesenteric (MBF), renal (RBF) and femoral (FBF) blood flows were made. Insulin sensitivity index (ISI) was calculated by the formula: ISI (dl/kg × min) = glucose infusion rate (mg/kg/min) ×

Table 1
Clinical and fasting biochemical parameters. Basal and after 2 year follow-up[a]

	BW (kg)	MAP (mmHg)	PG (mg/dl)	PI (μU/ml)	tChol (mg/dl)	TGs (mg/dl)
Control basal	13.4±1.8	96±8	95±8	10.1±3.9	102±19	27.4±6.1
24 months	14.9±1.8	106±6	97±8	8.2±1.7	99±17	29.2±8.0
Obese basal	13.5±1.9	94±6	97±6	9.3±3.2	98±22	28.6±4.9
24 months	21.7±3.3**	116±9*	114±9*	25.1±9.2*	201±33**	34.5±12.6
Capto basal	13.6±1.5	99±7	96±7	9.7±2.9	103±30	29.2±6.1
24 months	19.6±4.2*	109±7*†	109±7	14.2±9.6	195±75**	36.1±6.8
Prava+capto basal	13.8±1.9	98±5	97±3	9.6±3.4	101±20	30.1±5.4
24 months	16.6±2.1*†	110±6*†	107±6	13.5±8.1†	152±42*†‡‡	35.2±10.4

[a] BW: bodyweight; MAP: mean arterial pressure; PG: plasma glucose; PI: plasma insulin; tChol: total cholesterol; TGs: triglycerides. *$P < 0.05$ vs basal; **$P < 0.01$ vs basal; †$P < 0.05$ vs obese; ‡‡0.05 vs capto.

10^3/SSPG (mg/dl). Higher ISI indicates lower resistance to insulin-stimulated glucose uptake and vice versa.

Finally, as somatostatin (SS) was used to inhibit endogenous insulin release during the IST, the possible action of this peptide on the parameters under study was determined in a preliminary study. In this acute experiment ($n=5$) and after the same surgical procedure, the *per se* effects of somatostatin were characterized by infusing the same SS dose used in the IST, together with a low dose of insulin (0.1 mU/kg/min) to maintain basal insulinemia. Under this protocol, measurements of all the parameters were made in the basal state and during the SS infusion, but no significant change was observed except for the MBF which decreased significantly (unpublished data).

All data shown in the present paper for blood flow (_% BF) in response to the IST result from subtracting averaged percent changes obtained in the SS infusion protocol (_% BF_{SS}) from values obtained during the performance of the IST (_% BF_{IST}). By means of this calculation (_% BF = _% BF_{IST} − _% BF_{SS}), we consider that no effect attributable to SS can be considered in our study.

2.4. Analytical determinations

Biochemical determinations of plasma total cholesterol (tChol) and triglycerides (TG) were made by a Hitachi autoanalyzer (model 747) and plasma glucose (PG) levels were measured with a glucose analyzer (YSI 23A, Yellow Springs Instrument Co). Plasma insulin (PI) was quantitated by a commercial radioimmunoassay kit (Linco Research Inc., St Charles, MO).

2.5. Statistical analysis

Comparisons between periods within each group were performed by two-tailed paired *t* tests. Differences between the four groups were tested by ANOVA and the Bonferroni correction for multiple comparisons. A $P < 0.05$ was considered statistically significant. Values in the figures are expressed as mean ±SE, while in the Tables values are expressed as mean ±SD.

3. Results

3.1. Clinical and biochemical results after 2 year follow-up

Conscious dogs showed differences in the levels of clinical and biochemical parameters among groups, as depicted in Table 1. Animals on a high fat diet exhibited higher mean body weight values compared with

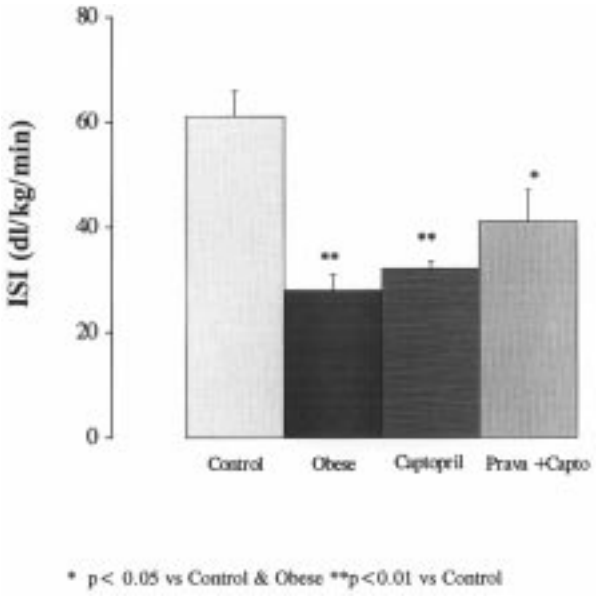

Fig. 1. Insulin sensitivity index after 2 years follow-up.

Table 2
Values of basal mean arterial pressure, mesenteric and renal blood flows, and their response to acute hyperinsulinism induced by an Insulin Suppression Test (IST)[a]

	Basal			IST			
Groups	MBF (ml/min)	RBF (ml/min)	MAP (mmHg)	MBF (Δ% vs Basal)	RBF (Δ% vs basal)	MAP (mmHg)	SSPI (μU/ml)
Control ($n=5$)	119±47.0	101±47	109±7	−3.2±1.6	−5.4±3.7	113±8	127±7.5
Obese ($n=5$)	115±35	109±55	111±8	−8.9±5.4	−7.2±4.6	118±9	125±23.6
Capto ($n=5$)	109±17	103±46	108±6	−7.1±4.9	−8.2±5.4	112±7	119±36.2
Prava+capto ($n=5$)	108±43	99±21	110±9	−2.9±2.6	−9.0±5.6	113±6	120±19.9

[a] MBF: mesenteric blood flow; RBF: renal blood flow; MAP: mean arterial pressure; SSPI: steady state plasma insulin.

the control group, although a significant difference was only attained in the obese and capto groups. The high fat diet was also associated with elevations in fasting plasma levels of glucose and triglycerides. Total cholesterol was elevated in the three groups receiving fat, but significantly lower values were exhibited by the prava+capto group. Finally, obese dogs showed increased blood pressure levels, while the blood pressures of treated dogs were comparable to control.

3.2. Basal blood flows and hemodynamic response to an IST

3.2.1. Insulin sensitivity index

In the present study the IST was designed to induce a hyperinsulinemic situation around the postprandial physiological range, with a normoglycemic state. The mean insulin level achieved during the tests (SSPI), ranged from 119 to 127 uU/ml, without differences between groups. Under these conditions, the three groups on high fat diet showed a decrease insulin sensitivity, estimated by a lower ISI compared with control group (Fig. 1). An intermediate degree of insulin resistance was observed in the prava+capto group vs control and obese groups.

3.2.2. Mean arterial pressure and mesenteric and renal flows, basal and during hyperinsulinemia

From a hemodynamic point of view, there were no differences in the basal levels of mesenteric and renal blood flows nor in mean arterial pressure. During hyperinsulinemic state, no differences between groups were observed (Table 2), although a slight trend of mean arterial pressure was observed in the obese group.

3.2.3. Femoral blood flow

As expression of skeletal blood flow, we used the measures in femoral bed. In basal situation (Fig. 2) significant differences were observed between captopril and obese groups versus control ($P < 0.05$–0.01), while prava+capto group showed significant differences versus obese group ($P < 0.05$). Systemic hyperinsulinemia induces a differential decrease in femoral flows versus basal situation that was significantly higher in obese

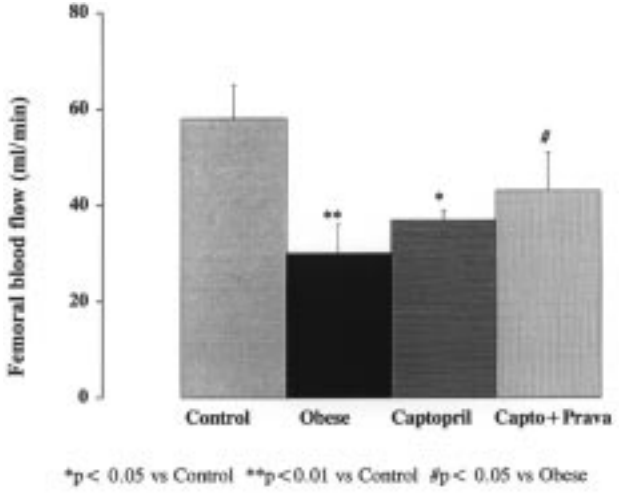

Fig. 2. Basal femoral blood flow after two years follow-up.

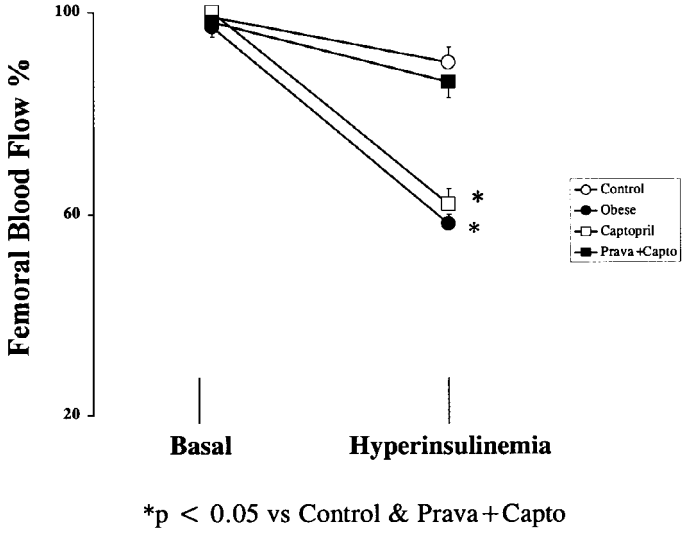

Fig. 3. Decrement (%) of femoral blood flow during hyperinsulinemia.

and capto groups, while the decrease of control and prava + capto groups do not attain significant differences (Fig. 3).

4. Discussion

Increasing evidence has accumulated during recent years favouring the hypothesis that insulin resistance, mainly in the obese syndrome, is associated with an endothelial dysfunction. This alteration could be an early and important feature in the development of vascular disease [20]. Our results suggest the possibility that this endothelial derangement, together with metabolic abnormalities linked to obesity, contribute to sustained reductions in peripheral blood flow.

In the present study we have demonstrated that peripheral vessels constitute preferential targets for vascular alterations associated with this long-term obesity model, as basal mesenteric and renal blood flows remained comparable in the control and obese groups, while the femoral blood flow was significantly reduced in the obese. These data are associated with an enhancement of the degree of insulin resistance in this group.

Obese humans have been shown to possess altered insulin-mediated and endothelium-dependent vasodilation [6,7]. Maximal expression of this peripheral alteration was detected in our obese group, as these dogs exhibited significantly higher reductions of FBF compared with control during the IST.

Various pathogenic factors, such as hypercholesterolemia, hyperinsulinemia, and hypertension, can independently and/or synergistically contribute to this defective hemodynamic response. Hypercholesterolemia, which was present in our fat-fed dogs, has been associated with abnormal endothelium-dependent vasorelaxation in human and experimental in vivo studies [21–23], and the implication of nitric oxide in this abnormality has been suggested [22,24]. In vitro experiments have confirmed that hypercholesterolemia inhibits production or bioactivity of nitric oxide [25,26]. A hyperlipidemic situation can be associated with a decrease in peripheral blood flow because skeletal muscle lipoprotein lipase activity, a main determinant of triglyceride uptake, is closely related to skeletal muscle fibre composition [27] and thereby blood flow. On the other hand, reduction of serum cholesterol has rendered positive effects in reversing impaired endothelium-dependent vasodilation in humans and in animals [16,28,29]. Our results are in agreement with these previous reports, as prava + -capto dogs, with lower cholesterol values, showed significantly higher basal levels of FBF, and minor reductions of this flow during the hyperinsulinemic period compared with obese dogs.

Hyperinsulinemia is another possible mechanism involved in the vascular alterations leading to blood flow changes. Overwhelming evidence has been accumulated during recent years on the acute vasodilator effect of insulin in the peripheral vasculature of healthy individuals [30]. This vasodilating role has been attributed to nitric oxide activity [31,32], a fact confirmed by a recent study demonstrating insulin's ability to stimulate endothelial NO production [33]. However, a large body of evidence has pointed out that this relaxing action is blunted in pathological states of insulin resistance associated with long-term exposure to hyperinsulinemia and also with endothelial dysfunction. In this respect, insulin stimulates various vasoconstrictor mechanisms that can elevate the vascular tone. For example, insulin induces sympathetic overactivity, with increased secretion of norepinephrine [34], enhances intracellular free calcium concentration [35], and elevates expression and circulating levels of endothelin-1 [36]. Furthermore, hyperinsulinemia, either directly or mediated by IGF-1 receptors, stimulates vascular smooth muscle cell hypertrophy and hyperplasia [37,38] and raises arterial stiffness [39]. Under these circumstances, in the obese syndrome these vasoconstrictor and trophic effects will not be counterbalanced by the vasodilatory effects of insulin, as a sustained defective NO production/release is associated with this disease. In this manner, altered endothelial function may contribute to the establishment of decreased peripheral blood flow, which could play an important role in the process of insulin resistance by limiting capillary recruitment and thereby reducing glucose uptake and metabolism [30]. Captopril has been reported to improve insulin sensitivity in humans [12] and obese dogs [40]. This beneficial action of CEIs seems to be related to a non-deleterious effect on the lipid profile, together with a vasodilatory action on peripheral blood flow which might be mediated by reduction of sympathetic activity [40] and stimulation of bradykinin production and stability [41,42]. In addition, improvement of endothelium-dependent vasodilation and prevention of structural changes in the vascular wall have been documented with CEIs in animal models [41,43] as well as in humans [44,45]. Our data showed a positive trend in the capto group for most metabolic and hemodynamic parameters. Furthermore, in agreement with our results, captopril therapy has been shown to reduce fasting insulin levels [11], which can also play a role in the prevention of long-term trophic effects of the hormone. Another mechanism by which captopril can exert a beneficial action in obesity is by reducing blood pressure and the trophic effects of angiotensin II.

In summary, in this chronic (2-year) model, obesity induces a higher degree of fasting hyperinsulinemia, deranged lipid profile, and lower ISI, that were ac-

companied by lower skeletal blood flow and an insulin-mediated femoral vasoconstriction. Combined therapy with captopril and pravastatin preserved hindlimb skeletal muscle blood flow and attenuated the insulin-mediated vasoconstriction observed in this model. This positive outcome might be related to synergistic effects of both drugs reducing the cardiovascular risk factors (hypertrophic and atherogenic) associated with this severe obesity model.

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