Reduction of Capillary Permeability in the Fructose-Induced Hypertensive Rat

Mouna Chakir, Gérard E. Plante, and Pierre Maheux

Impaired insulin transcapillary transport and the subsequent decrease in insulin delivery to target organs have been suggested to play a role in insulin resistance. These defects were studied in fructose-fed rats, an animal model with insulin resistance. For this study, male Sprague-Dawley rats were fed with either a 60% fructose enriched (F) or a standard chow diet (N) for a total of 2, 4, or 8 weeks. Capillary permeability to albumin was assessed at the end of each dietary period by quantifying the extravasation of albumin-bound Evans blue (EB) dye in different organs. Unanesthetized animals were injected with Evans blue dye (20 mg/kg) in the caudal vein 10 min before being killed and EB dye was extracted by formamide from selected organs collected after exsanguination. As expected, rats had an increase in blood pressure upon feeding with fructose at 4 and 8 weeks (F, 149 ± 3 mm Hg; N, 139 ± 3 mm Hg; $P < .05$). Using this technique, we showed a 56% and a 51% reduction in capillary permeability in skeletal muscles at 4 and 8 weeks of fructose feeding, respectively (4 weeks: N, 44.5 ± 5.0 μg/g of dry tissue; F, 19.8 ± 4.2 μg/g of dry tissue; $P < .01$ and 8 weeks: N, 23.3 ± 3.7 μg/g of dry tissue; F, 11.3 ± 4.0 μg/g of dry tissue; $P < .05$). Similar changes were observed at 4 weeks in the thoracic aorta (N, 82.8 ± 8.8 μg/g of dry tissue; F, 53.0 ± 5.1 μg/g of dry tissue; $P < .02$) and skin (N, 36.0 ± 5.3 μg of dry tissue; F, 15.0 ± 2.3 μg/g of dry tissue; $P < .02$) and at 8 weeks in the liver (N, 107.5 ± 4.3 μg/g of dry tissue; F, 80.9 ± 3.2 μg/g of dry tissue; $P < .01$). In conclusion, fructose feeding is accompanied by a significant and selective reduction of Evans blue leakage primarily in skeletal muscle and liver, and transiently in the skin and aorta, consistent with a role for decreased tissue insulin delivery in insulin resistance. Am J Hypertens 1998;11:563–569 © 1998 American Journal of Hypertension, Ltd.

KEY WORDS: **Insulin, fructose-fed rat, Sprague Dawley rat, capillary permeability, insulin resistance.**

There is accumulating evidence that insulin and insulin resistance have, in addition to their effects on glucose metabolism, significant cardiovascular effects. Insulin increases skeletal muscle blood flow and these effects appear to be reduced in humans with increasing levels of resistance to insulin.[1,2] Rasio et al[3] and Ader and Bergman,[4] and Steil et al[6] have also suggested that interstitial transfer of insulin from the capillary lumen to target cells could be an important determinant of peripheral glucose disposal in diabetic and nondiabetic individuals. Indeed, polypeptide hormones such as insulin need to diffuse or to be transported across the endothelial barrier for their action on skeletal muscle or liver. Vascular dysfunction, and decreased delivery of insulin to target organs as a result of impaired transcapillary transport of insulin, have been suggested to play a role in insulin resistance. However, endothelial permeability has not been well studied in

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TABLE 1. TISSUE CONTAMINATION BY RESIDUAL BLOOD DURING EVANS BLUE EXTRAVASATION STUDIES

	^{51}Cr Red Blood Cells (CPM/mg of tissue)	Relative Values (%)
Whole blood	29.2 ± 1.8	100
Lung parenchyma	0.9 ± 0.2	3.1
Heart	1.9 ± 0.2	6.5
Liver	2.2 ± 0.2	7.5
Kidney	1.4 ± 0.2	4.8
Skeletal muscle	0.4 ± 0.1	1.4
Skin	1.1 ± 0.3	3.8
Thoracic aorta	2.7 ± 0.3	9.2

Mean ± SEM (n = 6).

Relative contamination for each tissue was obtained by dividing the tissue count/minute (CPM) measured per milligram of tissue by the radioactive count measured in 1 mL of blood (or approximately 1 mg). Total count for blood is given per milliliter of blood.

human beings or animal models with insulin resistance. The purpose of this study was therefore to examine the capillary permeability characteristics of various organs in the fructose-fed rat, an established animal model of insulin resistance. To our knowledge, this is the first time that regional capillary permeability has been studied in an animal model with insulin resistance.

MATERIALS AND METHODS

General Protocol Male Sprague-Dawley rats (Charles River Breeding Laboratories, St.-Constant, Québec, Canada), initially weighing between 200 and 220 g, were used for these experiments. These animals were housed in temperature and humidity controlled conditions and were fed standard rat chow (Ralston Purina Canada, Woodstock, Ontario, Canada), containing 53% vegetable starch, 4.5% fat, 22% protein, 0.36% sodium, and 1.08% potassium prior to dietary manipulation. Rats were then randomly assigned to a standard chow diet or a 60% fructose enriched diet (Harlan Teklad, Madison, WI) for a total of 2, 4, or 8 consecutive weeks.

Blood Pressure Measurement The tail cuff method was used to measure the systolic blood pressure. Ambient temperature was maintained at 30°C. The equipment used included magnetic tail holders connected to a manual scanner, a pulse amplifier, and a dual channel recorder. The systolic blood pressure was measured in an unanesthetized state. The mean of four consecutive readings was used as the measurement of the systolic blood pressure of each rat.

Measurement of Capillary Permeability The Evans blue (EB) dye technique was used to measure capillary permeability to albumin on day 14 (2 weeks), 28 (4 weeks), or 56 (8 weeks) in selected tissue obtained from unanesthetized control and experimental rats. This technique has been described in several papers[6–8] and is a reliable estimate of the extravasation and interstitial accumulation of albumin.[9] Briefly, rats were injected with EB dye (20 mg/kg; Sigma, St. Louis, MO) in the caudal vein, 10 min before being killed and exsanguinated. The thorax was cut and the lungs perfused with 15 mL of Krebs solution (10 mL/min) via the pulmonary artery to remove intravascular dye. Specimens from intrathoracic, splanchnic, and peripheral organs were obtained: skeletal muscle (left quadriceps), thoracic aorta, skin, heart, kidneys, liver, and lungs. Tissues were weighed immediately. Half of each tissue sample was dried at 60°C for 24 h, and a dry/wet weight ratio was calculated to avoid underestimation of Evans blue dye concentration due to local edema. The other half was placed in a formamide solution (4 mL/g wet tissue) for 24 h for dye extrac-

TABLE 2. ANIMAL CHARACTERISTICS AND METABOLIC DATA AT THE END OF EACH DIETARY TREATMENT PERIOD

	Dietary Treatment Periods		
	Week 2	Week 4	Week 8
Group size			
Normal chow	5	8	8
Fructose	6	9	9
Body weight (g)			
Normal chow	328 ± 7	390 ± 6	467 ± 17
Fructose	312 ± 3	370 ± 8	457 ± 16
P value*	NS	NS	NS
Systolic BP (mm Hg)			
Normal chow	139 ± 5	146 ± 3	131 ± 3
Fructose	148 ± 6	157 ± 3	141 ± 3
P value	NS	<.04	<.05
Plasma glucose (mmol/L)			
Normal chow	9.3 ± 0.9	8.0 ± 0.2	9.9 ± 0.5
Fructose	7.7 ± 0.6	9.0 ± 0.3	9.7 ± 0.7
P value	NS	<.03	NS
Plasma insulin (pmol/L)			
Normal chow	507 ± 111	614 ± 80	586 ± 45
Fructose	476 ± 72	889 ± 78	818 ± 86
P value	NS	<.03	<.02
Plasma triglycerides (mmol/L)			
Normal chow	0.5 ± 0.1	1.9 ± 0.2	1.7 ± 0.4
Fructose	2.5 ± 0.5	5.0 ± 0.5	6.8 ± 1.3
P value	<.01	<.01	<.01

Mean ± SEM (range in parentheses).

** Mann-Whitney U rank sum test for each dietary period; NS, not statistically significant (P > .05); BP, blood pressure.*

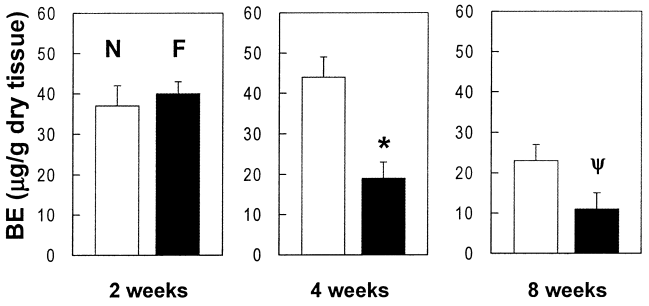

FIGURE 1. *Extravasation of Evans blue dye (BE) in skeletal muscles (quadriceps) of Sprague-Dawley rats fed with a normal chow (N; open bar) or a fructose-enriched diet (F; solid bar) for 2, 4, and 8 consecutive weeks.* *P < .01, ΨP < .05.

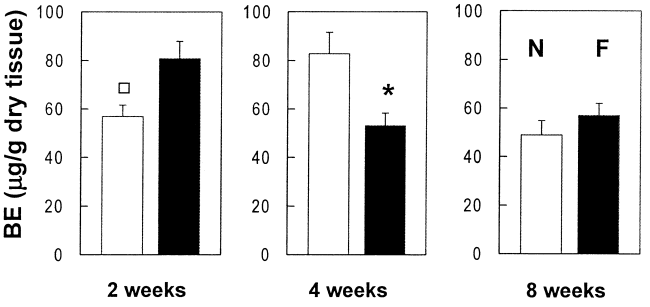

FIGURE 2. *Extravasation of Evans blue dye (BE) in the thoracic aorta of Sprague-Dawley rats fed with a normal chow (N; open bar) or a fructose-enriched diet (F; solid bar) for 2, 4, and 8 consecutive weeks.* P < .05, *P < .02.

tion. The extracted amount of Evans blue dye was determined by spectrophotometry at 620 nm using a 96-well microplate photometer (Titertek Instruments, Huntsville, AL). Concentration of EB dye in each tissue was expressed in micrograms/gram of dry tissue to avoid underestimation due to tissue edema. Complete and tight EB dye binding of serum albumin, extracellular equilibration of the marker, as well as entire extraction of the dye by formamide were all recently validated in our own,[6,7] and other laboratories.[10] Results were calculated from a standard curve of Evans blue dye (0.5 to 25 μg/mL) and expressed as Evans blue dye micrograms per gram of dry weight of tissue. The remote possibility of incomplete removal of the vascular EB dye by exsanguination of animals would not appear to affect the absolute contents of the dye, assumed to be distributed in the interstitia of the selected organs, as the contribution of the vascular space of these organs represents < 10% of the total tissue volume.[11] Using ^{51}Cr-labeled erythrocytes, the maximal contamination of tissue samples obtained after exsanguination under the above described conditions was also <10% of the measured intravascular values (Table 1). Finally, feeding experiments with normal or fructose chow were always conducted in parallel to avoid seasonal changes of the microvascular permeability.

Biochemical Measurements Caudal vein blood samples were taken at the end of each dietary treatment period. Food was removed at 09:00, the rats were weighed, and blood was taken by tail bleeding at 13:00. Blood samples were centrifuged, aliquoted, frozen, and later assayed for glucose, insulin, and triglyceride concentrations.

Statistical Analyses Data are reported as mean values ± SEM. Significance was determined using the Mann-Whitney rank sum test because several variables were not normally distributed and some experiments included a small number of animals. $P < .05$ was considered statistically significant.

RESULTS

Weight and Metabolic Changes Table 2 illustrates the effects of the fructose-enriched diet versus the normal chow diet on body weight, blood pressure, and plasma glucose, insulin, and triglyceride concentrations. These results demonstrate that weight gain was similar in both groups over the series of dietary protocols. As expected, fructose feeding was associated with a small but significant increase in tail systolic blood pressure at 4 and 8 weeks of dietary manipulations. Plasma glucose concentrations were generally comparable in each dietary period, with the exception of a statistically significant increase at 4 weeks ($P < .03$). This suggests an association between fructose feeding and some degree of glucose intolerance. An increase in plasma insulin concentration began to develop at week 4 in the fructose-treated group as compared with the normal chow-treated group.

Measurements of Microvascular Permeability Figures 1, 2, 3 and Table 3 compare the quantitative extravasation of Evans blue dye in selected organs of chow- fed as well as fructose-fed animals. It can be

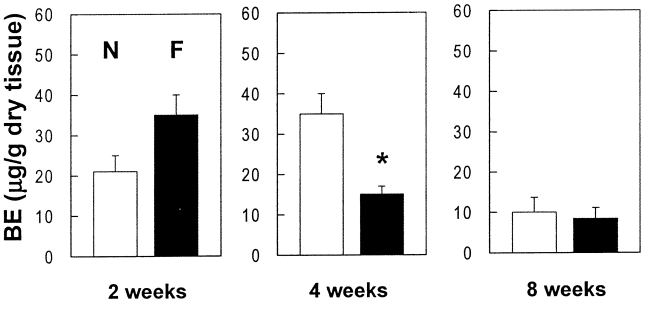

FIGURE 3. *Extravasation of Evans blue dye (BE) in the skin of Sprague-Dawley rats fed with a normal chow (N; open bar) or a fructose-enriched diet (F; solid bar) for 2, 4, and 8 consecutive weeks.* *P < .02.

TABLE 3. COMPARISON OF EXTRAVASATION OF EVANS BLUE DYE IN MILLIGRAMS/DRY TISSUE WEIGHT IN SELECTED ORGANS OF ANIMALS FED A FRUCTOSE-ENRICHED OR A NORMAL CHOW DIET FOR 2, 4, OR 8 CONSECUTIVE WEEKS

Organ	Dietary Treatment Periods		
	Week 2	Week 4	Week 8
Skeletal muscle			
Normal chow	37.2 ± 12.1 (6.0–78.5)	44.5 ± 5.0 (16.1–61.7)	23.3 ± 3.7 (8.6–40.1)
Fructose	39.7 ± 2.6 (32.8–50.0)	19.8 ± 4.2 (2.9–45.6)	11.3 ± 4.0 (0.0–30.9)
P value*	NS	<.01	<.05
Thoracic aorta			
Normal chow	56.8 ± 4.8 (39.4–66.7)	82.8 ± 8.8 (42.7–115.7)	48.8 ± 6.0 (19.3–69.2)
Fructose	80.8 ± 7.1 (49.0–99.8)	53.0 ± 5.1 (26.0–76.1)	56.9 ± 5.1 (29.3–76.5)
P value	<.05	<.02	NS
Skin			
Normal chow	21.0 ± 4.1 (7.0–30.2)	36.0 ± 5.3 (9.5–56.5)	10.3 ± 3.7 (0.0–21.2)
Fructose	34.8 ± 4.7 (17.6–52.4)	15.0 ± 2.3 (4.9–28.1)	8.4 ± 2.8 (0.0–22.1)
P value	NS	<.02	NS
Heart			
Normal chow	95.8 ± 4.1 (83.9–107.6)	109.1 ± 5.9 (79.4–132.3)	103.3 ± 4.6 (72.5–114.4)
Fructose	115.3 ± 7.3 (90.0–135.0)	118.3 ± 4.9 (93.0–143.5)	111.4 ± 6.2 (82.7–134.2)
P value	NS	NS	NS
Kidneys			
Normal chow	135.6 ± 7.1 (112.4–155.9)	138.0 ± 7.6 (92.1–159.7)	114.0 ± 11.1 (78.6–178.3)
Fructose	138.0 ± 11.5 (96.0–165.3)	155.2 ± 10.1 (108.9–193.4)	99.1 ± 6.4 (75.3–140.8)
P value	NS	NS	NS
Liver			
Normal chow	87.9 ± 8.2 (65.9–111.5)	101.9 ± 5.7 (63.2–114.3)	107.5 ± 4.3 (89.6–129.7)
Fructose	95.1 ± 3.5 (85.4–105.7)	102.3 ± 2.2 (93.3–115.8)	80.9 ± 3.2 (67.9–97.9)
P value	NS	NS	<.01
Lungs			
Normal chow	41.8 ± 3.1 (31.2–49.3)	62.0 ± 4.1 (44.1–76.5)	72.0 ± 11.1 (33.3–111.2)
Fructose	69.9 ± 7.5 (54.8–104.6)	84.2 ± 13.9 (34.6–184.1)	51.1 ± 5.4 (18.5–64.3)
P value	<.02	NS	NS

Mean ± SEM (range in parenthesis).

** Mann-Whitney U rank sum test for each dietary period; NS, not statistically significant (*P > .05*).*

seen from these data that there was an alteration in the distribution pattern of albumin-bound Evans blue dye in the fructose fed animals. Notably, there was a marked reduction in skeletal muscle permeability at 4 and 8 weeks of feeding with fructose (Figure 1). More specifically, Evans blue dye extravasation was lower at 4 weeks in fructose fed animals (19.8 ± 4.2 μg/g of dry tissue) as compared with the normal chow-fed animals (44.5 ± 5.0 μg/g of dry tissue), illustrating a statistically significant 56% reduction ($P < .01$). Similarly, the same phenomenon was observed in skeletal muscles at 8 weeks of diet being 23.3 ± 3.7 μg/g of dry tissue in normal chow-fed animals and 11.3 ± 4.0 μg/g of dry tissue in fructose-fed animals (−51%; $P < .05$). There was also statistically significant reductions of capillary permeability in the thoracic aorta and skin at 2 and 4 weeks of fructose feeding; these changes were not sustained after week 8 of the dietary manipulations (Figures 2 and 3). Interestingly, we also observed a reduction in the extravasation of Evans blue dye in the liver after 8 weeks of fructose feeding (−25%; $P < .01$, Table 3). Finally, measurements of Evans blue dye extravasation in other organs such as heart, kidneys, and liver were not associated with any significant changes. No change was seen in the pulmonary airways or in the gastrointestinal tract (data not shown).

DISCUSSION

Insulin resistance is recognized as a considerable metabolic cardiovascular risk factor as well as a prevalent problem in non–insulin-dependent diabetes mellitus.[12,13] This latter entity is clearly associated with endothelial cell dysfunction, which is thought to play a central role in the pathogenesis of hypertension, retinopathy, microalbuminuria, and atherosclerosis.[14–17] The role of capillary endothelial cell dysfunction in the development of target organ damage has been studied extensively in different animal models of diabetes and insulin resistance.[18] These studies have

suggested that vascular dysfunction is present long before the appearance of frank diabetes mellitus. Because the study of these phenomena in human beings is complicated by the inaccessibility of viable and representative vascular tissue, we need to rely on different animal models to make inferences regarding human diseases.

The present data have shown, in fructose-fed rats, that insulin resistance is associated with significant and very selected alterations in vascular permeability to albumin. This animal model, in addition to being hypertriglyceridemic, shows some important components of the human Syndrome X such as hypertension, hyperinsulinemia, insulin resistance, and impaired glucose tolerance.[19,20] Microvascular permeability and mechanisms underlying the elevation of blood pressure in this model remains unclear. However, our data have shown that fructose feeding is accompanied by a selective reduction of Evans blue dye leakage in skeletal muscle, skin, aorta, and liver. These observations suggest that permeability properties of capillaries differ greatly among organs; however, only changes in skeletal muscles and liver seemed to be chronic.

These very selective changes in regional capillary permeability can theoretically result in an alteration of fluid distribution as well as a different access of small soluble molecules to target tissues. We suggest that access of insulin and glucose to insulin-dependent tissue such as the skeletal muscle or liver is important in insulin resistance states. Skeletal muscle is one of the most important organs for glucose disposal, being responsible for 75% of total body glucose disposal in humans under hyperinsulinemic conditions. Therefore, a reduction in the permeability of capillary membranes to insulin and further dilution of the hormone in the extravascular matrix can reduce the final concentration of insulin at a cellular level or in the immediate environment of a target cell. Although this suggestion does not minimize in any way the role of insulin postreceptor phenomena, it is likely that impaired access of insulin to target tissues contributes to a reduction in the insulin-mediated glucose disposal known in the rodent model.

Our microcapillary assessment technique uses Evans blue dye, which avidly binds to the negatively charged intravascular albumin. This technique is a reliable way of assessing transvascular fluxes of macromolecules.[21] Even though albumin is a fairly large and slowly exchanging macromolecule (83 kDa), it is possible to hypothesize that transcapillary transport of insulin, a protein of approximately 6 kDa, could also be impaired. Although the passive permeation of metabolites across capillaries is affected by molecular size, hydrophilic molecules such as glucose or insulin can also cross the capillary wall via paracellular water-filled channels and pores. The final concentration of insulin reaching, for example, a muscle cell, is determined by the permeability of capillary membranes to insulin, and by its dilution in the extravascular space. Although transport of albumin could be different from the one used by insulin and glucose, the observation that Evans blue dye leakage is reduced in this insulin resistance animal model remains of interest. It should be acknowledged that some of these in vivo Evans blue dye measurements were variable with coefficients of variation ranging from 6% to 100% for some tissues. However, it is also crucial to realize that these experiments were performed in unanesthetized animals and that other variables such as plasma insulin or triglyceride concentrations had coefficients of variation that were also as high as 61%. The fact that these latter biological variables were also variable and that significant differences were observed using nonparametric statistical tests (such as the Mann-Whitney rank sum test) suggests that large coefficients of variation in Evans blue dye tissue concentrations was not a simple function of insensitive methodology.

The reduction of skeletal muscle capillary permeability is a particularly important observation in insulin resistance, as this organ has a very high capillary density that can accommodate large increases in the transcapillary transfer rate of oxygen and, in the event of exercise, can increase its insulin and non–insulin mediated glucose uptake. Skeletal muscle also uses 20% to 25% of total cardiac output.[22] Taking this into account, this large vascular bed is likely to have a substantial effect on total body glucose disposal as well as on systemic blood pressure. It is of particular interest to note the concurrent increase in blood pressure and change in muscle permeability, evident after 4 weeks fructose feeding, which were documented in the present experiments. This intriguing phenomenon suggests that alterations in muscle capillary permeability precede the appearance of hypertension. Despite the fact that the number of animals studied after 2 weeks of fructose was small, and that this could have prevented the detection of an increase in blood pressure, the observed relationship remains extremely interesting.

Rasio et al,[3] Ader and Bergman,[4] and Steil et al[5] have shown that transcapillary insulin transport is a sluggish process that could have an important influence on the time course of insulin in vivo. However, to our knowledge, this phenomenon lacks complete validation in diabetes and other insulin resistant states.

Of primary interest to the authors of this article is that these observations were very different from what our group showed in two other animal model studies. Indeed, Chakir et al have shown that capillary permeability was increased in streptozotocin-induced diabetic[23] and in spontaneously hypertensive rats.[7] Although this discrepancy cannot be explained, we sug-

gest that the hyperglycemia of the former and the very high blood pressure of the latter could contribute to the increased permeability. Diabetes is classically characterized by an increase in capillary permeability;[14–18] however, other studies have demonstrated that diabetes is associated with an increased microvascular reactivity.[24,25]

Theoretically, a reduction in EB dye extravasation in selected organs could be caused by different circulatory factors. First, capillary pressure and therefore transcapillary transport of molecules is in part controlled by the pre- to postcapillary resistance ratio, and thus the peripheral vasoconstriction associated with hypertension could itself contribute to the present findings. In the particular case of fructose induced insulin resistance, if precapillary resistance (terminal arterioles) is high, the precapillary pressure drops, and exchanges of solute across the microvascular bed could decrease. Alternatively, if the postcapillary resistance is reduced, the transit time of macromolecules is reduced, impeding transport of small molecules such as as insulin, glucose, or albumin. Finally, fructose feeding and insulin resistance can also impair the capillary membrane permeability itself or the complex biochemical structure of the underlying proteoglycan meshwork. In any of these scenarios, any one of these changes can result in an impaired supply of blood and substrates to important tissues such as skeletal muscle or liver. The experimental design of the present study did not allow a differentiation between these possibilities.

The pathophysiological mechanisms underlying the observed changes in EB extravasation are unknown. However, many mechanisms can be implicated. First, it should be remembered that the pre- to postcapillary resistance ratio is influenced by both the sympathetic nervous system and local factors. Secondly, in addition to its passive functions as a porous membrane, the endothelial barrier can synthesize various vasoactive substances that may impair the ability of vessels to fully dilate. Among these, endothelin-1 could be implicated. Because an increase in the latter could have been of interest to us, plasma concentrations of this hormone were measured, with no significant difference found between the fructose and the chow-fed animals (data not shown). Alternatively, the dilator response to several endothelium-dependent agonists has also been shown to be attenuated in diabetes;[1,2,15] it is therefore possible that this impairment is responsible for the observed reduction in capillary permeability. Finally, hyperinsulinemia could be involved in the underlying pathophysiology. Although insulin decreases systemic vascular resistance and increases skeletal muscle blood flow in nondiabetics, it has been shown recently that its capacity to increase blood flow, a phenomenon mediated through the nitric oxide pathway, was significantly reduced in conditions associated with insulin resistance such as obesity, hypertension, and non–insulin-dependent diabetes mellitus (NIDDM), as well as in animal infused for 72 h with insulin.[1,15] The roles of insulin resistance and hyperinsulinemia remain hypothetical, with more research required to establish the extent and significance of these different effects on capillary permeability.

In conclusion, these results suggest that there is a reduction in capillary permeability, mainly in skeletal muscle, upon feeding with fructose for 4 and 8 weeks. We suggest that this reduction in capillary permeability could reflect tissue specific endothelial dysfunction or be secondary to the peripheral vasoconstriction associated with the fructose-induced rise in blood pressure. This may contribute to abnormal internal distribution of fluids in body, reduced capillary delivery of insulin to target organs (such as muscle and liver), and perhaps hypertension.

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