Hemodynamic effects of lipids in humans

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Stojiljkovic, Milos P., Da Zhang, Heno F. Lopes, Christine G. Lee, Theodore L. Goodfriend, and Brent M. Egan. Hemodynamic effects of lipids in humans. Am J Physiol Regulatory Integrative Comp Physiol 280: R1674–R1679, 2001.—Evidence suggests lipid abnormalities may contribute to elevated blood pressure, increased vascular resistance, and reduced arterial compliance among insulin-resistant subjects. In a study of 11 normal volunteers undergoing 4-h-long infusions of Intralipid and heparin to raise plasma nonesterified fatty acids (NEFAs), we observed increases of blood pressure. In contrast, blood pressure did not change in these same volunteers during a 4-h infusion of saline and heparin. To better characterize the hemodynamic responses to Intralipid and heparin, another group of 21 individuals, including both lean and obese volunteers, was studied after 3 wk on a controlled diet with 180 mmol sodium/day. Two and four hours after starting the infusions, plasma NEFAs increased by 134 and 111% in those receiving Intralipid and heparin, P < 0.01, whereas plasma NEFAs did not change in the first group of normal volunteers who received saline and heparin. The hemodynamic changes in lean and obese subjects in the second study were similar, and the results were combined. The infusion of Intralipid and heparin induced a significant increase in systolic (13.5 ± 2.1 mmHg) and diastolic (8.0 ± 1.5 mmHg) blood pressure as well as heart rate (9.4 ± 1.4 beats/min). Small and large artery compliance decreased, and systemic vascular resistance rose. These data raise the possibility that lipid abnormalities associated with insulin resistance contribute to the elevated blood pressure and heart rate as well as the reduced vascular compliance observed in subjects with the cardiovascular risk factor cluster.

ELEVATED PLASMA NONESTERIFIED fatty acids (NEFAs) emerge as one potential link between insulin resistance and hypertension. Resistance to insulin’s NEFA lowering action is severely impaired in abdominally obese hypertensive patients and correlates with blood pressure (BP) independently of insulin and insulin-mediated glucose disposal (11). Patients with familial combined hyperlipidemia and their affected relatives have elevated plasma NEFAs (26) and higher BP (8). Moreover, plasma NEFAs measured after an overnight fast and 2 h after an oral glucose load independently predicted the development of hypertension (13).

From a mechanistic perspective, fatty acids can impair endothelial cell nitric oxide synthase activity and endothelium-dependent dilation in vitro (10). Endothelium-dependent vasodilation is impaired (32) and vascular α1-adrenoceptor-mediated responses are enhanced (19, 33, 35) when plasma NEFAs are raised in humans with Intralipid and heparin.

Experimental studies in animals support a link between NEFAs and hypertension. In minipigs, BP and vascular resistance in most tissue beds rise when plasma NEFAs are elevated during an infusion of Intralipid and heparin (6). Portal venous infusions of oleate induce a pressor response in rats mediated by α1-adrenoceptors (17). Obesity-induced hypertension in dogs is blocked by clonidine, a centrally acting sympatholytic (30), and combined α- and β-adrenocceptor blockade (20). In contrast, raising NEFAs in humans has either not changed (2, 28) or led to a modest 3–5% increase of systolic BP (32).

This paper addresses the hemodynamic effects of raising NEFAs with Intralipid and heparin in humans. The data suggest that lipid abnormalities associated with insulin resistance raise BP, heart rate, and vascular resistance, whereas small and large artery compliance decreases, at least in the short term.

METHODS

Human Volunteers

Thirty-two volunteers, 21–49 yr of age, were recruited by advertisement and paid. Before participation in the study, each subject signed an informed consent approved by the Office of Research Integrity and Risk Protection and the General Clinical Research Center Review Committee at the
Medical University of South Carolina. Subjects that provided informed consent had a history, physical examination, blood and urine laboratory tests, and an electrocardiogram.

Participants in phase 1 included 11 lean (body mass index (BMI) <25 kg/m²) volunteers with normal BP (<130/85 mmHg) and lipid profiles (cholesterol <200, triglycerides <150, high-density lipoprotein cholesterol (HDL-C) >45 mg/dl). Volunteers in phase 2 included nine obese (BMI ≥27 kg/m²) dyslipidemic (triglycerides ≥150 mg/dl, HDL cholesterol ≤45 mg/dl) subjects with high blood pressure to stage 1 hypertension (130–159/85–99 mmHg) and 12 lean normotensive subjects with normal lipid profiles. All subjects abstained from medications for 2 wk before beginning the study. Patients on treatment for hypertension discontinued medication for a 2-wk washout period. During this time, they monitored their BPs twice daily and recorded values in a diary. They were enrolled only if their BP remained within the range noted above.

Physiological Measurements

BP. During the screening and qualifying period, systolic and diastolic BP was determined by the first and fifth Korotkoff sounds, respectively. Values were obtained after 5 min of rest in the seated position from the right arm supported at heart level to the nearest 2 mmHg with a standard mercury sphygmomanometer. During the laboratory protocol, BP was measured on volunteers in the supine position using mercury sphygmomanometry.

Arterial compliance and systemic hemodynamics. The HDI/PulseWave Research CardioVascular Profiling Instrument model CR 2000 (Hypertension Diagnostics, Eagan, MN) was used to assess the arterial pulse wave (25). The tonometer was secured over the left radial artery, and the BP cuff was placed on the right upper arm. This instrument was used for estimating stroke volume, cardiac output, large and small artery elasticity, and systemic vascular resistance.

Biochemical Measurements

NEFAs. Blood for NEFAs was drawn into prechilled Eppendorf tubes containing disodium EDTA and paraoxon (Sigma Chemical, St. Louis, MO) to inhibit lipoprotein lipase and prevent hydrolysis of NEFAs from triglycerides in vitro (41). Plasma was stored at −70°C before analysis of total plasma NEFAs by the 63Ni method (4, 16).

Study Protocol

Phase 1. Volunteers consumed their usual diet before study. After an overnight fast, subjects came to the Clinical Physiology Laboratory in the outpatient General Clinical Research Center (GCRC) at 0800 on 2 separate days separated by at least 1 wk. Venous catheters were placed for the infusions and blood sampling. Twenty minutes after establishing venous access, baseline BP and heart rate were obtained in triplicate over 10 min. On one of the 2 study days, subjects were infused with saline (0.8 ml·m⁻¹·min⁻¹) and heparin (200 U bolus, followed by 1,000 U/h), whereas on the other study day, subjects received the same volume of 20% Intralipid and heparin. BP and heart rate were recorded three times at baseline and once every 30 min after the start of the infusion. Blood for NEFAs was drawn at baseline and at 2 and 4 h after starting the infusion.

Phase 2. Patients were on an isocaloric diet, which averaged ~2,000 kcal/day. The composition of the diet consisted of 210 g carbohydrates, 90 g fat, 80 g proteins, 180 mmol NaCl, and 50 mmol K⁺ each day for 3 wk. Fresh fruits and vegetables were limited, and tea and supplemental vitamins were not permitted. Thus the diet was relatively low in antioxidants. After completing 21 days on the diet, subjects were admitted to the outpatient GCRC following an overnight fast. After placement of intravenous catheters and a 30-min adaptation period, baseline hemodynamic data were obtained, and blood was drawn for the metabolic assays. Subjects then received a 4-h-long infusion of 20% Intralipid (Baxter Healthcare, Glendale, CA) at 0.8 ml·m⁻¹·min⁻¹ and heparin (200 U bolus, followed by 1,000 U/h). Heparin was given to activate lipoprotein lipase and to accelerate the hydrolysis of fatty acids from triglycerides (34). BP, heart rate, and arterial compliance were recorded in triplicate at baseline and every 30 min during the infusion. Blood samples for NEFAs were obtained at baseline and again at 2 and 4 h after starting the infusion.

Data Analysis

Data are presented as means ± SE. The data were analyzed using SPSS 10.0 software (SPSS, Chicago, IL). Statistical methods included t-tests for the paired data and repeated-measures ANOVA for the time-series variables. The graphic displays of the data were created using Sigma Plot 2000 (SPSS). P values <0.05 were accepted as statistically significant.

RESULTS

The initial pilot study in 11 lean normotensive volunteers showed that systolic and diastolic BP increased by 10 ± 2 and 3 ± 2 mmHg, respectively, during a 4-h-long infusion of Intralipid and heparin. A 4-h-long infusion of saline and heparin in these subjects did not induce any significant change in systolic or diastolic BP.

Changes in plasma NEFA concentrations during the infusions of saline and heparin (phase 1) and Intralipid and heparin (phase 2) are shown in Fig. 1. The Intralipid and heparin infusion raised plasma NEFA concentrations by 134% at 2 h and 111% at 4 h. The NEFA values during the infusion were significantly increased by 10.2 ± 0.3 μmol/L. The changes in plasma NEFA concentrations during the infusions are shown in Fig. 1. The Intralipid and heparin infusion raised plasma NEFA concentrations by 134% at 2 h and 111% at 4 h. The NEFA values during the infusion were significantly increased by 10.2 ± 0.3 μmol/L.
greater than baseline as well as the values observed during the control saline and heparin infusion. In contrast, the infusion of saline and heparin did not significantly change plasma NEFAs compared with baseline.

Changes in systolic and diastolic BP and heart rate were similar in the lean and obese volunteers, so the results were combined. As shown in Fig. 2, the Intralipid and heparin infusion resulted in significant increases in all three variables, especially during the final 2 h of the infusion. At the end of the 4-h infusion, systolic (13.5 ± 2.1 mmHg) and diastolic (8.0 ± 1.5 mmHg) BP increased, and heart rate rose (9.4 ± 1.4 beats/min).

The data on arterial compliance and systemic vascular resistance during the Intralipid and heparin infusion are provided in Fig. 3. Small and large artery elasticity (compliance) decreased by 45 and 30%, respectively, during the infusion of Intralipid and heparin, whereas systemic vascular resistance increased.

After a small initial increase, stroke volume tended to decline. Thus the increase of cardiac output during the Intralipid and heparin infusion resulted from the increase of heart rate (data not shown).

DISCUSSION

The infusion of Intralipid and heparin, which reproduces lipid abnormalities characteristic of the insulin resistance syndrome, significantly raises BP and heart rate in human volunteers (Fig. 2). Small and large artery compliance fall, and systemic vascular resistance rises (Fig. 3). These data raise the possibility that lipid abnormalities associated with insulin resistance, e.g., increased NEFAs and/or triglycerides, contribute to the elevated BPs associated with this syndrome.

Previous studies in humans showed that NEFAs had vascular effects, which could elevate BP. More specifically, local and systemic vascular responses to phenylephrine, an α₁-adrenoceptor agonist, were enhanced when plasma NEFAs locally were raised in healthy volunteers to levels observed in obese, insulin-resistant subjects (19, 33). This effect of NEFAs could contribute to the increased neurovascular reactivity and tone reported in overweight, hypertensive patients (35). Moreover, elevating plasma NEFAs in normal volunteers impaired regional endothelium-dependent vasodilation (31, 32).

These observations in humans are consonant with earlier studies in animals that showed that raising NEFAs systemically in minipigs induced a substantial rise of BP and vascular resistance (6). Moreover, infusing oleic acid into the portal veins of rats induced a significant rise of BP, which was blocked by α₁-adrenoceptor antagonists (17). Rats fed a high-fat diet had an increase in plasma NEFAs and a 12-mmHg rise in systolic BP (36).

In contrast to the clear findings in short-term experimental studies in animals, substantial and consistent increases of BP have not been reported in humans when plasma fatty acids were raised acutely in the

Fig. 2. Change from baseline in systolic blood pressure (ΔSBP), diastolic BP (ΔDBP), and heart rate (ΔHR) in subjects infused with Intralipid and heparin (■, n = 21) or with saline and heparin (○, n = 11). *P < 0.05 compared with baseline; †P < 0.05 compared with saline and heparin data.
laboratory. In one study, systolic BP rose by 3–5% when plasma NEFAs were raised with Intralipid and heparin (32). Although most of the Intralipid and heparin infusions were limited to 1–2 h, one protocol continued the infusion for 10 h without observing a significant change of BP (2). Consequently, this is the first study to document that raising NEFAs with Intralipid and heparin can induce substantial changes in several hemodynamic variables including BP in humans.

The explanation for the greater effects of Intralipid and heparin on BP in this study compared with previous reports in humans is not clear. Because the preparation and instrumentation for these studies are relatively extensive, BP of the volunteers may rise during this time. If a stable baseline BP is not established, then an increase of BP during the Intralipid and heparin infusion could be counterbalanced by the return of BP to baseline over time. Our volunteers appear to have reached their true baseline, because BP did not change during the control infusion of saline and heparin.

Another plausible explanation for the greater pressor effect of Intralipid and heparin in our volunteers could be related to regional differences in nutrition. Fatty acids activate a protein kinase C-dependent increase in the production of reactive oxygen species, i.e., oxidative stress (22, 23). Oxidative stress, in turn, has been linked to hypertension (24) and vascular remodeling (12, 18, 22, 23). The antioxidant capacity of volunteers, which has several nutritional determinants, may be lower among subjects living in the Southeast U.S. compared with volunteers in other areas (9, 21, 27). Thus the oxidative stress induced by the rise of NEFAs during the Intralipid and heparin infusion may have been greater in our volunteers compared with subjects in the other reports. In fact, the diet consumed by subjects in phase 2 of this study was relatively low in antioxidants. Fresh vegetables, fruits, and fruits juices were limited, and tea and supplemental vitamins were not permitted. Their diet was low in antioxidants. Fresh vegetables, fruits, and fruits juices were limited, and tea and supplemental vitamins were not permitted. Their diet was low in antioxidants. Thus oxidative stress during the infusion of Intralipid and heparin may have been greater in our volunteers compared with subjects in previous studies, which could explain their greater BP responses.

The findings of the present study are consistent with a large body of evidence that implicates the dyslipidemia of insulin resistance in the pathogenesis of hypertension in humans. More specifically, subjects with familial combined hyperlipidemia appear strongly predisposed to hypertension (37–39). These patients and their affected first-degree relatives manifest several features of the insulin-resistance syndrome including higher plasma NEFAs, triglycerides, and BP, especially the systolic values (8). Among a group of abdominally obese hypertensives and normotensives, resistance to suppression of plasma NEFAs concentrations and turnover during euglycemic hyperinsulinemia correlated with BP independently of measures of hyperinsulinemia and insulin-mediated glucose disposal (11). Moreover, the Paris Prospective Study demonstrated that plasma NEFAs measured after an overnight fast and 2 h after an oral glucose load were significant and independent predictors for the development of hypertension in normotensive, nondiabetic men (13).
Systolic BP tended to rise more than diastolic BP during the infusion of Intralipid and heparin (Fig. 2). Systolic BP has been linked more closely with abnormalities of arterial compliance, whereas diastolic BP has been associated more closely with vascular resistance (29). Thus the disparate increase of systolic and diastolic BP in this study may coincide with the greater reduction in arterial compliance compared with the rise of vascular resistance (Fig. 3).

This study was not designed to determine the mechanisms underlying the reduction of arterial compliance during the infusion of Intralipid and heparin. However, in normal volunteers infused with the competitive inhibitor of nitric oxide synthase, L-N^G^-monomethyl-L-arginine, arterial compliance declined (15). In that study, the reduction of small artery compliance was greater than the decline in large artery compliance, and this suggests that the former is more dependent on nitric oxide. Fatty acids impair nitric oxide synthase activity and impair endothelium-dependent vasodilation (10, 31). Thus the decline of arterial compliance, with relatively greater effects on small rather than large arteries, may have reflected an adverse effect of NEFAs on nitric oxide and endothelium-dependent vascular tone.

Although the increase of BP during the infusion of Intralipid and heparin may be attributed to peripheral vascular effects of NEFAs, the data indirectly implicate a central neurogenic component. More specifically, if the short-term rise of BP was mediated only by peripheral vascular effects, then an increased stretch of arterial baroreceptors should have raised vagal tone and reduced heart rate (14). However, heart rate increased significantly along with the increase of BP. Thus the elevation of both BP and heart rate suggests that the short-term dyslipidemia altered central autonomic control of the cardiovascular system. Additional studies are required to elucidate this point. Nevertheless, the evidence for a neurogenically mediatedpressor response to the short-term (4 h) infusion of Intralipid and heparin is consonant with several other reports. Obese, insulin-resistant humans have elevated BP and heart rate as well as increased sympathetic nervous system activity (5). BPs and heart rates fall more in obese than in lean hypertensive patients treated with combined α1- and β-adrenergceptor blockade (40). Heart rates and BP both rise along with multiple metabolic and neuroendocrine markers with obesity-induced hypertension in animals (1, 3, 7, 20, 30). Obesity-induced hypertension in dogs is prevented by clonidine (30), a central sympatholytic, and by blockade of both α1- and β-adrenergceptors (20).

**Perspectives**

Insulin-resistant subjects manifest a dyslipidemia characterized by elevated plasma NEFA and triglyceride concentrations. These patients tend to have higher BPs and heart rates as well as lower arterial compliance than healthy individuals. The infusion of Intralipid and heparin, which raises plasma fatty acids and triglycerides, increases systolic and diastolic BP, raises heart rate, and decreases arterial compliance. Our findings raise the possibility that lipid abnormalities observed among insulin-resistant subjects contribute to their elevated BPs and vascular pathology.

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