20-HETE and Circulating Insulin in Essential Hypertension With Obesity

Cheryl L. Laffer, Michal Laniado-Schwartzman, Alberto Nasjletti, Fernando Elijovich

Abstract—Analogous to observations in Dahl salt-sensitive (SS) rats, we have shown that 20-hydroxyeicosatetraenoic acid (20-HETE) is involved in the pathogenesis of SS essential hypertension. A strong negative correlation between urine 20-HETE and body mass index (BMI) remains unexplained. We measured BP, urine sodium (UNaV), and 20-HETE in obese hypertensive subjects during a 24-hour salt load (160 mmol NaCl diet ± 2 L intravenous saline). We classified them into insulin-resistant (IR) (n=14) and insulin-sensitive (IS) (n=12), with the average insulin sensitivity index (SI=22.5×[fasting glucose×insulin]⁻¹) of 3 days (cutoff for IR, SI <0.161 mL · L/μU · mmol). IR were older (50±1 versus 44±2, P<0.03), more obese (BMI 38.2±1.4 versus 32.0±1.5 kg/m², P<0.01), and had higher insulin (39.2±2.3 versus 22.0±1.1 μU/mL, P<0.0001) and lower SI (0.084±0.009 versus 0.222±0.013, P<0.0001) than IS. Blood pressure, UNaV, and sodium balance did not differ between groups. SI correlated negatively with age (r=−0.39, P<0.05) and BMI (r=−0.53, P<0.01). Urine 20-HETE was less in IR than in IS when normalized by serum insulin (0.91±0.25 versus 2.24±0.46 μg · 24 hours⁻¹/μU · mL⁻¹, P<0.02), but not if uncorrected. Urinary 20-HETE excretion correlated negatively with insulin (r=−0.40, P<0.04), whereas the relationship between 20-HETE and SI was not statistically significant. Our data suggest that increased circulating insulin, not the state of insulin resistance, suppresses urine 20-HETE excretion in obese hypertensive subjects. Findings in experimental models suggest that an inhibitory effect of insulin on cytochrome P4504A, rather than effects of insulin on membrane-bound arachidonic acid or on its release to the cytosol, may explain our observation. (Hypertension. 2004;43[part 2]:1-5.)

Key Words: hypertension ▪ obesity ▪ arachidonic acid ▪ insulin ▪ insulin resistance

In addition to renal prostaglandins generated via the COX pathway, other eicosanoids that inhibit tubular sodium transport are produced by CYP 450 monoxygenase metabolism of arachidonic acid (AA). 20-Hydroxyeicosatetraenoic acid (20-HETE) is the major compound produced by this pathway in the mammalian kidney. 20-HETE inhibits proximal tubular and, most importantly, loop of Henle sodium transport. A deficit of renal medullary 20-HETE plays a role in determining increased TAL chloride transfer, shifted pressure–natriuresis, salt-sensitivity of blood pressure, and salt-dependent hypotension in the Dahl salt-sensitive (SS) strain. 5–9

In human essential hypertension, we have shown that 20-HETE excretion (1) is regulated by salt intake; 10 (2) relates differently with the natriuresis of a salt-load in SS versus salt-resistant subjects; 10 (3) participates in the natriuretic action of furosemide, an inhibitor of the Na⁺K⁺2Cl⁻ cotransporter of the loop of Henle; 11 and (4) responds to this diuretic in a manner positively related to furosemide-induced natriuresis and negatively related to salt-sensitivity of BP. 11 These observations support physiological and pathophysiological roles for 20-HETE in regulation of natriuresis and BP in humans.

In the preceding studies, we also observed a strong negative relationship between the excretion of 20-HETE and body mass index (BMI), 10 suggesting that a factor related to obesity might be responsible for decreased synthesis or excretion of this eicosanoid in hypertension. However, a mechanism for this observation was not apparent in our studies.

The insulin-resistant phenotype is commonly observed in normotensive obese, 12 hypertensive lean, 13 hypertensive obese, 14 and SS hypertensive subjects. 15 Therefore, in this study we set out to investigate whether relationships exist between circulating insulin or insulin sensitivity and urine excretion of 20-HETE in a group of overweight or obese essential hypertensive patients.

Methods

Twenty-six subjects with BP >140 mm Hg systolic or >90 mm Hg diastolic or who were receiving antihypertensive therapy were recruited at the University of Texas Medical Branch (UTMB). A diagnosis of essential hypertension and a BMI greater than 25 kg/m² were required for inclusion in the study. The research was approved by the Institutional Review Board, and all subjects gave informed consent. Patients receiving antihypertensive drugs discontinued them for at least 2 weeks, and all subjects were instructed to maintain their usual diet for 2 weeks before study. All patients provided demo-
graphic and clinical data and had a physical examination, periodic BP measurements, and blood counts, chemistries, serum creatinine, electrolytes, and an EKG performed for monitoring of safety.

Subjects were then admitted to the Clinical Research Center to determine their individual insulin sensitivity and, during a 24-hour period of salt loading, to measure urine 20-HETE, urine Na excretion, and BP. Salt was administrated as a 160-mmol NaCl diet prepared in a metabolic kitchen and a 2-L intravenous infusion of normal saline, administered from 8:00 AM to 12:00 PM (noon).

Insulin sensitivity was estimated using the Homeostatic Model Assessment method ($\text{SHOMEA}_3$) in which sensitivity index (SI) = $0.161 \times \text{glucose}^{-0.25} \times \text{insulin}^{1.25}$, PFB-trimethylsilyl-20-HETE was incubated with bistrimethylsilyl-trifluoroacetic acid for 2 hours at 37°C to obtain a second derivative, PFB-trimethylsilyl-20-HETE-PFB-TMS constructed by NCI-GC/MS analyzer. Concentration in the samples was in the ng/mL range and was multiplied by total 24-hour urine volume to express results as daily excretion.

The method for measurement of 20-HETE has been previously described in detail.10 In brief, deuterated 20-HETE was used as internal standard. Samples were incubated with Escherichia coli β-glucuronidase (Sigma Chemical Co) for 2 hours at 37°C. After initial extraction and thin-layer chromatography steps, 20-HETE in the extracts was converted to the pentfluorobenzyl-bromide derivative (20-HETE-PFB). After a second thin-layer chromatography, 20-HETE-PFB was incubated with bis(trimethylsilyl) trifluoroacetamide to obtain a second derivative, PFB-trimethylsilyl-20-HETE (20-HETE-PFB-TMS). Total 20-HETE in the purified samples was estimated by comparison of ion intensities at m/z 391:393 versus a standard curve of the molar ratio 20-HETE-PFB-TMS/20-20-deuterated-HETE-PFB-TMS constructed by NCI-GC/MS analysis. Concentration in the samples was in the ng/mL range and was multiplied by total 24-hour urine volume to express results as daily excretion rates (µg/24 hours).

Data are presented as means ± SEM. Comparisons between IR and insulin-sensitive (IS) patients were made with unpaired t tests. Correlation coefficients were calculated with Pearson method. These tests and single linear regression analyses were performed with JMP software (version 3.0.2; SAS Institute). P<0.05% was used to reject the null hypothesis.

Results

Subjects were 47±1 years old, with similar representation of blacks (n=15) and whites (n=11); 77% were female. Average BMI was 35.3±1.2 kg/m² (range: 26.5 to 50.7), with 85% exceeding 30 kg/m², which is the currently accepted cutoff for the definition of clinical obesity. The Table shows that IR subjects were older and more obese than their IS counterparts. Other clinical characteristics, BP, sodium excretion, and the positive sodium balance achieved during the salt-load did not differ between the two groups. In contrast, serum insulin was significantly higher and SI was significantly lower in IR than in IS subjects, reflecting the cutoff used for definition of these groups.

Figure 1 shows significant negative correlations of SI with BMI and age. That is, the greater the obesity of individual patients or the older their age, the greater was the magnitude of their insulin resistance. Analogous to our previous observations, 20-HETE excretion had a significant positive correlation with urine sodium excretion during the salt load (r=0.42, P<0.04) and a negative correlation with BMI (r=-0.42, P<0.04) not shown.

Figure 2 (left panel) shows that urine excretion of 20-HETE, expressed in absolute values, was slightly lower in IR (39.9±7.8) than in IS (46.7±8.0 µg/24 hours) subjects. However, this difference did not reach statistical significance because of an outlier observation: the IR patient with the SI closest to the cutoff had a 20-HETE value of 2.2 SDs above the mean, ie, beyond the maximum 95% confidence limits for the group (Figure 2). In contrast, the right panel of Figure 2 shows that 20-HETE excretion was significantly less in IR than in IS subjects (0.91±0.25 versus 2.24±0.46 µg·24 hours⁻¹µU⁻¹ ml⁻¹; P<0.02) when 20-HETE excretion data were normalized by the serum insulin concentration of each patient.

Figure 3 shows a negative, statistically significant correlation between urine 20-HETE and circulating insulin. That is, the higher the levels of serum insulin, the lower the levels of

<table>
<thead>
<tr>
<th>Variable</th>
<th>IR (n=14)</th>
<th>P</th>
<th>IS (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>50±1</td>
<td>&lt;0.03</td>
<td>44±2</td>
</tr>
<tr>
<td>Race (W/B)</td>
<td>6/8</td>
<td>ns</td>
<td>5/7</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>11/3</td>
<td>ns</td>
<td>9/3</td>
</tr>
<tr>
<td>BP (mm Hg)</td>
<td>153±6/88±3</td>
<td>ns</td>
<td>148±3/91±2</td>
</tr>
<tr>
<td>UNaV (mmol/24 h)</td>
<td>326±35</td>
<td>ns</td>
<td>337±28</td>
</tr>
<tr>
<td>Na balance (mmol/24 h)</td>
<td>134±35</td>
<td>ns</td>
<td>123±28</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>38.2±2.4</td>
<td>&lt;0.01</td>
<td>32.0±1.5</td>
</tr>
<tr>
<td>Fasting insulin (µU/mL)</td>
<td>39.2±2.3</td>
<td>&lt;0.0001</td>
<td>22.0±1.1</td>
</tr>
<tr>
<td>SI (ml·L/µU·mmol)</td>
<td>0.084±0.009</td>
<td>&lt;0.001</td>
<td>0.222±0.013</td>
</tr>
</tbody>
</table>

IR indicates insulin-resistant; IS, insulin-sensitive; W, white; B, black; BP, average blood pressure from the ambulatory monitor the day of the salt-load; UNaV, urine sodium excretion on the same day; BMI, body mass index; SI, insulin sensitivity index; ns, not statistically significant.

Figure 1. Relationships of insulin sensitivity index (SI) with age (left) and BMI (right) in the group as a whole. Correlation coefficients and their statistical significance are indicated on the graphs.
urine 20-HETE. This relationship was present only when all patients were analyzed together, not if IR and IS subject results were analyzed separately. In contrast, an apparent positive relationship between 20-HETE excretion and SI index was of borderline statistical significance ($r=0.33$, $0.1>P>0.05$; not shown).

**Discussion**

A role for a deficiency of 20-HETE in Dahl SS hypertension has been firmly established. Our studies on urinary excretion of this eicosanoid in humans have provided indirect evidence for an analogous role in human hypertension. The present studies were conducted to explore the mechanism of an intriguing and unexplained observation of our previous research: a strong negative correlation between urine 20-HETE excretion and BMI in essential hypertensive subjects.

Because obesity with or without hypertension is linked to insulin resistance, and because the latter is a phenotype also observed in lean human hypertension and SS hypertension, we hypothesized that excretion of 20-HETE in hypertensive subjects might be associated either with insulin resistance or with hyperinsulinemia.

Our data support the latter of the two possibilities. The classification of patients in dichotomous manner (IR and IS) using an arbitrary cutoff for insulin sensitivity derived from maximum normal glucose and insulin levels did not produce striking results. That is, 20-HETE excretion, albeit somewhat lower in IR patients, was not significantly diminished in this group (unless data were normalized by insulin levels) and exhibited high variability in both. Similarly, the relationship between the SI index and 20-HETE excretion in all patients was of borderline significance. In contrast, the main observation of our study was the statistically significant negative relationship between serum insulin and 20-HETE when the data were analyzed as a continuous set in the entire population. The latter observation suggests that hyperinsulinemia, although reflecting the insulin resistant state, is a better correlate of 20-HETE excretion in humans compared with IR itself.

There are no human data to speculate about possible mechanisms for our findings. In insulin resistance, lean and obese Zucker rats, a model for diabetes type 2 and obesity, arachidonic/oleic acid ratios in skeletal muscle and heart are diminished, an alteration that can be improved by increasing insulin sensitivity with the peroxisome proliferator DHEA. That is, the content of muscle AA, as a proportion of total phospholipids, correlates with tissue insulin sensitivity. If diminished availability of AA caused by insulin resistance were the factor determining diminished 20-HETE production in our obese hypertensive patients, the negative relationship between 20-HETE and insulinemia would merely reflect the circulating insulin response to the insulin resistant state, which is not consistent with our results.

It is conceivable that a direct effect of insulin on lipid metabolism decreases AA substrate availability to the CYP450 system, as suggested by the finding that insulin infusion reduces circulating AA in fetal sheep. However, most of the evidence in tissue preparations is opposite to this observation. For example, AA is diminished in sarcolemmal membranes, isolated testicular cells, and peritoneal polymorphonuclear leukocytes of streptozotocin-diabetic, insulin-deficient rats. These animals exhibit impaired conversion of cis 8,11,14 icosatetraenoic acid to AA, perhaps because of deficiency of desaturases. These abnormalities are corrected partially or completely by insulin via an increase in tissue uptake of the cis 8,11,14 icosatetraenoic acid precursor. Thus, insulin-induced decreases in AA availability are an extremely unlikely explanation for our results.

Insulin could impair synthesis of 20-HETE via inhibition of PLA2, the major enzyme responsible for hydrolysis of membrane phospholipids and release of AA substrate to the cytosol. Whether insulin is an inhibitor of PLA2 is controversial, particularly because the actions of insulin on this lipase seem to be tissue- and isoform-dependent. Hence, in insulin-deficient streptozotocin-diabetic rats, striated and cardiac muscle PLA2 activity is increased, a change that is reversed by insulin, indicating an inhibitory action of the hormone on the lipase. In contrast, there is no action of insulin on secretory PLA2 mRNA in osteoblasts or on the activity of PLA2 in rat liver microsomes. Moreover, renal glomerular cytosolic PLA2 is actually increased in hyperinsulinemic OLEFT rats. Therefore, there is no clear-cut experimental evidence to support the possibility that insulin-
induced inhibition of PLA2 activity could participate in reduced synthesis of 20-HETE by human kidney.

Finally, our findings could be explained by a direct inhibitory action of insulin on the CYP450 enzymatic complex. Effects of insulin, insulin deficiency, and hyperinsulinemia on CYP mRNA expression, protein content, and activity have been studied in diverse experimental models. Results in liver and kidney of streptozotocin-treated mice and rats,31 liver microsomes of Gold Syrian hamsters, obese Zucker rats, and streptozotocin-treated Wistar rats,32,33 and in rat hepatocyte cultures exposed to insulin,34 indicate that the predominant effect of insulin on the CYP450 system is in fact inhibitory, whereas that of insulin deficiency is stimulatory. However, there is some variation in results for the different families of CYP450 proteins. Seemingly opposite results (eg, induction of CYP3A4 in cultured rat and human hepatocytes by the insulin sensitizer troglitazone35 and stimulation of CYP1A2 in liver microsomes of rats with insulinoma transplants36) may be attributed to pharmacological effects of the thiazolidinediones or to supraphysiological insulin levels, respectively. Interestingly, the effect of insulin is inhibitory on CYP4A1, CYP4A2, and CYP4A3 in liver and kidney microsomes of the rat.37 These oxidases are precisely those responsible for 20-HETE synthesis in rats and belong to the same family as one of the two major CYP isoforms that synthesize 20-HETE in humans, CYP4A11.38 Therefore, it is theoretically possible that the negative relationship between circulating insulin and urine 20-HETE excretion that we observed in our patients could be caused by insulin-induced suppression of CYP450 activity with consequent reduction of renal synthesis of 20-HETE.

Regardless of its mechanism, a relationship between hyperinsulinemia and a major regulator of sodium transport and BP has obvious implications for hypertension in obesity. Our studies suffer from the limitations imposed by conducting non-invasive research in humans. That is, we do not know whether urine excretion of 20-HETE reflects its renal synthesis, and, if so, by which compartment. The latter is important because the vasoconstrictor and transport actions of this compound may be differentially exerted in the renal cortex and medulla. Notwithstanding this, the potential importance of our studies should stimulate further research in non-human models in which renal tissue components of the CYP450 system can be measured.

Perspectives

Obesity is currently an epidemic in the United States and other parts of the world. The prevalence of hypertension in the United States is increasing for the first time in 4 decades.39 Therefore, unraveling the mechanisms of hypertension in obesity has become of utmost importance. Our observations suggest the possibility that the obese phenotype of insulin resistance exerts, via hyperinsulinemia, inhibitory actions on a major regulator of natriuresis, 20-HETE, the most abundant product of monooxygenation of AA by CYP450 enzymes. The importance of confirming and expanding our observations in humans and other models stems from the fact that insulin resistance and CYP450 activity can be pharmacologically manipulated. We propose that combined insulin-sensitization with thiazolidinediones and stimulation of CYP450 expression by fibrin acid derivatives may constitute a specific antihypertensive therapy in obese essential hypertensive subjects.

Acknowledgments

This work was supported by MO1 RR00073 NCRR (C.L.L., F.E.) and NIH PO1 34300 (A.N., M.L.S.).

References


20-HETE and Circulating Insulin in Essential Hypertension With Obesity
Cheryl L. Laffer, Michal Laniado-Schwartzman, Alberto Nasjletti and Fernando Elijovich

Hypertension. published online January 5, 2004;
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/early/2004/01/05/01.HYP.0000112224.87290.3a.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/