Non-esterified fatty acids increase arterial pressure via central sympathetic activation in humans

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ABSTRACT

Previous studies have shown that acute increases in plasma NEFAs (non-esterified fatty acids) raise SVR (systemic vascular resistance) and BP (blood pressure). However, these studies have failed to distinguish between CNS (central nervous system) mechanisms that raise sympathetic activity and paracrine mechanisms that increase SVR directly, independent of CNS involvement. The aim of the present study was to directly determine whether the sympathetic nervous system contributes to the pressor response to NEFAs. On 2 days separated by at least 2 weeks, 17 lean healthy volunteers (ten male/seven female; age, 22 ± 1 years; body mass index, 23 ± 1 kg/m²; values are means ± S.E.M.) received a 4-h intravenous infusion of 20% Intralipid® or placebo (in a single-blind randomized balanced order). MSNA (muscle sympathetic nerve activity), HR (heart rate), BP (oscillometric brachial measurement) and Q (cardiac output; acetylene rebreathing) were measured before and throughout infusion. The change in HR (+8.2 ± 1.0 and +2.4 ± 1.2 beats/min), systolic BP (+14.0 ± 1.6 and +3.2 ± 2.5 mmHg) and diastolic BP (+8.2 ± 1.0 and −0.1 ± 1.7 mmHg) were significantly greater after the 4-h infusion of Intralipid® compared with placebo (P < 0.001). The change in BP with Intralipid® resulted from an increase in SVR (Q/mean arterial pressure; P < 0.001) compared with baseline, without a change in Q. MSNA burst frequency increased during Intralipid® infusion compared with baseline (+4.9 ± 1.3 bursts/min; P < 0.05), and total MSNA (frequency × amplitude) was augmented 65% (P < 0.001), with no change during placebo infusion. Lipid infusion increased insulin, aldosterone and F2-isoprostane, but not leptin, concentrations. On the basis of the concomitant increase in BP, MSNA and SVR, we conclude that central sympathetic activation contributes to the pressor response to NEFAs.

INTRODUCTION

Accumulating evidence links elevated plasma NEFA (non-esterified fatty acid) levels with insulin resistance and hypertension, two components of the metabolic syndrome. Epidemiological research indicates that elevated fasting NEFA concentrations are a strong independent predictor for the development of hypertension [1]. Levels of NEFAs in obese hypertensive patients are approximately twice that measured in lean normotensive

Key words: blood pressure, fat emulsion, fatty acid, hypertension, insulin, sympathetic nerve activity.

Abbreviations: AngII, angiotensin II; BP, blood pressure; CBF, calf blood flow; CVR, calf vascular resistance; DBP, diastolic BP; HR, heart rate; I/H, Intralipid®/heparin; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; NA, noradrenaline; NEFA, non-esterified fatty acid; POMC/CART, pro-opiomelanocortin/cocaine- and amphetamine-regulated transcript; Q, cardiac output; S/G, saline/glycerol; SBP, systolic BP; SNS, sympathetic nervous system; SV, stroke volume; SVR, systemic vascular resistance; TAG, triacylglycerol; VO2max, maximal oxygen uptake.

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individuals [2], and the ability of insulin to lower NEFAs is severely impaired in these patients. Moreover, NEFA concentrations correlate directly with BP (blood pressure) measured at rest and over 24 h, independently of insulin and insulin-mediated glucose disposal [3].

Studies over the past decade have examined whether acutely elevating the NEFA concentration increases BP, and the underlying mechanisms associated with the pressor response to NEFAs. For example, I/H (Intralipid®/heparin) infusion in minipigs significantly augmented BP and vascular resistance in most tissue beds [4]. Likewise, portal and femoral venous infusions of oleic acid induced a pressor response in rats [5,6], and I/H infusion in humans increased BP and resistance in most [7–9], but not all [10], investigations.

Despite the emerging literature linking NEFAs to metabolic abnormalities and hypertension, there is a paucity of results regarding the role of the SNS (sympathetic nervous system) in the pressor response to NEFAs. This is surprising given the evidence that some forms of hypertension with obesity and high-fat diets may be neurogenically mediated [11,12]. Studies of various lengths in normotensive rats and humans have shown that acutely elevating the NEFA concentration increases [5,8], decreases [13] or does not change [14] sympathetic activity. However, probably due to indirect measures of sympathetic activity [i.e. NA (noradrenaline) concentrations] [5,8,13] or insufficient duration of previous studies [14], it remains unclear whether NEFAs activate the SNS.

Therefore the main aim of the present study was to investigate the role of NEFAs on central sympathetic activation in humans by direct measurement of MSNA (muscle sympathetic nerve activity) for a sufficient duration to establish a complete temporal response. A secondary aim was to determine whether leptin and/or insulin are plausible mediators of the sympathetic response to NEFAs. Finally, we sought to fill the gaps of previous studies by measuring various hormonal [e.g. aldosterone and AngII (angiotensin II)] and haemodynamic variables, together with MSNA, in the framework of a randomized blinded placebo-controlled design. We hypothesized that NEFAs would augment MSNA and BP, and that this response would be associated with increases in insulin and leptin.

MATERIALS AND METHODS

Subjects
A total of 17 young (18–31 years of age) subjects participated in the present study (Table 1). All women were tested during days 2–7 of the follicular phase of their menstrual cycle [15]. Each subject underwent a complete medical screening, including DXA (dual energy X-ray absorptiometry) and a graded exercise test for determination of \( V_{\text{O}_2,\text{max}} \) (maximal oxygen uptake).

Table 1 Characteristics of the study subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (n) (male, female)</td>
<td>10, 7</td>
</tr>
<tr>
<td>Age (years)</td>
<td>22 ± 1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173 ± 0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69 ± 3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23 ± 1</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.88 ± 0.01</td>
</tr>
<tr>
<td>( V_{\text{O}_2,\text{max}} ) (ml · kg⁻¹ · body weight · min⁻¹)</td>
<td>45 ± 2</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>155 ± 6</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>53 ± 3</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>86 ± 5</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.73 ± 0.11</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.43 ± 0.01</td>
</tr>
</tbody>
</table>

All subjects were healthy, active, but not athletically trained, normotensive, non-diabetic, non-obese and non-smokers who were not taking any medication, including oral contraceptives. Subjects abstained from alcohol for 2 days and caffeine for 1 day prior to the experiment. Approval was obtained from the Institutional Review Board of the Pennsylvania State University. Each subject gave written informed consent, and all procedures conformed to the Declaration of Helsinki.

Instrumentation
The study utilized a cross-over design involving two identical experimental days (differing only in the substance infused), separated by a period of at least 2 weeks. The infusion of I/H or S/G (saline/glycerol) was randomized, balanced and single-blinded. All subjects consumed a standardized meal of 54.4 kJ/kg of body weight, consisting of 55% carbohydrate, 30% fat and 15% protein, between 18.00 and 20.00 hours on the night prior to the experiment. After an overnight fast, subjects reported to the laboratory (approx. 07.00 hours) and lay in a supine position on a bed. A 20-gauge catheter was inserted into one antecubital vein for infusion, and an 18-gauge catheter was placed retrograde into the contralateral dorsal hand vein for blood sampling. This arm was kept on a heating pad to arterialize the venous blood. Subjects were then instrumented to measure HR (heart rate), BP, CBF (calf blood flow), \( Q \) (cardiac output) and MSNA.

Measurements
HR and arterial pressure
HR (three-lead ECG) was measured and recorded continuously. Oscillometric brachial BP (Colin 7000) was measured after a 30-min period of rest (twice at
1-min intervals) and every 10 min throughout infusion. MAP (mean arterial pressure) was estimated as diastolic pressure + 1/3 pulse pressure.

**CBF**

CBF was determined by venous occlusion plethysmography on the calf [16]. During each data-recording period, blood flow was attained with two sets of measurements (separated by 4 min each) consisting of four cycles in succession. CVR (calf vascular resistance) was estimated as corresponding brachial MAP/CBF.

\[ Q = \frac{Q}{MAP} \]

\[ SV = \frac{Q}{HR} \]

\[ SVR = \frac{Q}{MAP} \]

\[ Q = \text{stroke volume} \]

\[ MAP = \text{mean arterial pressure} \]

\[ HR = \text{heart rate} \]

**MSNA**

Peroneal MSNA was recorded as described previously [18]. Briefly, a tungsten electrode (FHC) was inserted into the peroneal nerve of the leg. Raw recordings were amplified, filtered and full-wave rectified to produce a recording of 'integrated' MSNA. Nerve traffic was expressed both as bursts/min and as bursts/min × mean burst amplitude, an index of total MSNA.

**Protocol**

Following instrumentation and a 30-min adaptation period, baseline haemodynamic and sympathetic data were obtained, and blood was sampled for metabolic and hormonal assays. Subjects then received a 4-h infusion of either I/H [20% Intralipid® (0.8 ml · m⁻² · min⁻¹; Baxter) [7] and heparin (200 unit bolus, followed by 0.3 unit · kg⁻¹ of body weight · min⁻¹)] or S/G [sham infusion, 2.25% glycerol/saline (New England Compounding), infused at the same rate and volume as I/H]. Heparin was infused with Intralipid® to activate lipoprotein lipase and to accelerate the hydrolysis of NEFAs and glycerol was drawn into pre-chilled tubes containing EDTA and 0.275 g of paraoxon/l (Sigma) to inhibit lipoprotein lipase and to prevent hydrolysis of NEFAs from TAG in vitro [20]. Samples for catecholamines were collected in pre-chilled tubes containing EGTA and glutathione. Serum insulin and leptin concentrations were determined by ELISA. Plasma NEFA and TAG concentrations were determined by a colorimetric method. Glycerol was measured enzymatically, catecholamines by HPLC, and aldosterone and AngII by RIA. F₂-isoprostanes were determined by immunoassay.

**Statistical analysis**

Recordings of MSNA and HR (5 min of data) were collected at baseline and every 30 min during infusion; each 5-min period was averaged to a single value. CBF was measured eight times, and was measured in duplicate at baseline and every 30 min during infusion; likewise, data from each period were averaged to a single value.

A repeated-measures ANOVA was used to detect treatment effects on neural, hormonal and haemodynamic variables. Least-squares means with Bonferroni correction were performed when appropriate to identify where differences between treatments occurred. Linear least-squares fit regression was conducted to examine the relationship between MSNA and CVR. The level of significance was set at \( P = 0.05 \). Values are means ± S.E.M. All calculations were made using SAS 9.1 software.

**RESULTS**

**Anthropometric, metabolic and hormonal measurements**

All subjects were young, healthy, normotensive, non-obese and were not insulin-resistant (Table 1). No gender differences in any responses were detected, so data from men and women were pooled for analysis. Infusions of I/H were well-tolerated by all of the subjects.

Plasma NEFA, TAG, glycerol and glucose concentrations during the infusions of S/G and I/H are shown in Table 2. Infusion of I/H was associated with a significant rise in plasma NEFAs and TAG compared with baseline and S/G infusion, whereas no changes occurred with S/G infusion. Plasma glycerol increased with S/G infusion and to a greater extent during I/H infusion. No significant changes in plasma glucose concentrations occurred with either day.

Serum leptin and insulin, and plasma adrenaline concentrations are shown in Figure 1, and plasma aldosterone, AngII and F₂-isoprostane concentrations...
Table 2  Time course of plasma NEFA, TAG, glucose and glycerol concentrations during infusion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Infusion</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEFAs (μmol/l)</td>
<td>I/H</td>
<td>502 ± 52</td>
<td>1419 ± 51*†</td>
<td>2104 ± 142*†</td>
<td>2301 ± 93*†</td>
<td>2266 ± 110*†</td>
</tr>
<tr>
<td></td>
<td>S/G</td>
<td>502 ± 52</td>
<td>518 ± 50</td>
<td>556 ± 50</td>
<td>617 ± 36</td>
<td>657 ± 38</td>
</tr>
<tr>
<td>TAG (mmol/l)</td>
<td>I/H</td>
<td>0.87 ± 0.07</td>
<td>3.03 ± 0.13*†</td>
<td>4.17 ± 0.30*†</td>
<td>4.99 ± 0.43*†</td>
<td>5.76 ± 0.54*†</td>
</tr>
<tr>
<td></td>
<td>S/G</td>
<td>0.79 ± 0.07</td>
<td>0.97 ± 0.07</td>
<td>0.94 ± 0.06</td>
<td>0.97 ± 0.07</td>
<td>1.00 ± 0.07</td>
</tr>
<tr>
<td>Glycerol (μmol/l)</td>
<td>I/H</td>
<td>72 ± 9</td>
<td>774 ± 44*†</td>
<td>1072 ± 61*†</td>
<td>1239 ± 81*†</td>
<td>1288 ± 101*†</td>
</tr>
<tr>
<td></td>
<td>S/G</td>
<td>79 ± 7</td>
<td>239 ± 10*</td>
<td>244 ± 12*</td>
<td>243 ± 16*</td>
<td>262 ± 16*</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>I/H</td>
<td>4.45 ± 0.10</td>
<td>4.69 ± 0.07</td>
<td>4.50 ± 0.09</td>
<td>4.35 ± 0.09</td>
<td>4.46 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>S/G</td>
<td>4.52 ± 0.10</td>
<td>4.52 ± 0.12</td>
<td>4.38 ± 0.13</td>
<td>4.57 ± 0.13</td>
<td>4.55 ± 0.12</td>
</tr>
</tbody>
</table>

are shown in Table 3. Baseline hormone concentrations were similar in both experimental conditions. The leptin concentration decreased during both infusions; however, the treatment × time interaction (P = 0.053) suggested a tendency for NEFAs to maintain leptin levels compared with the decline in leptin with placebo. The insulin concentration rose significantly by 3 and 4 h of I/H infusion, but did not increase in response to S/G infusion. Infusion of I/H significantly lowered plasma NA concentrations by 3 and 4 h, whereas NA increased by the final hour of S/G infusion. When expressed as a change from baseline (Figure 2), the NA concentration was unchanged with I/H infusion; however, a significant treatment interaction (P = 0.001) was still present. Both F2-isoprostanes and aldosterone increased during I/H infusion, whereas aldosterone significantly decreased during S/G administration. No changes in AngII and adrenaline concentrations were observed with either I/H or S/G infusion (Table 3).

Haemodynamic measurements

As shown in Table 3, I/H infusion was associated with a significant increase in SBP (systolic BP, +14.0 ± 1.6 compared with +3.2 ± 2.5 mmHg), DBP (diastolic BP, +8.2 ± 1.0 compared with −0.1 ± 1.7 mmHg), and HR (+8.2 ± 1.0 compared with +2.4 ± 1.2 beats/min) compared with S/G infusion (P < 0.001), especially during the last 2 h of infusion. Despite the increase in HR with I/H infusion (Figure 3), Q remained unchanged due to a decrease in SV, which was not different compared with the S/G infusion day. Paralleling the increase in MSNA, with I/H infusion SVR was significantly elevated compared with baseline (P < 0.001) at 4 h, but did not change during S/G infusion. Although CBF (Figure 2) increased significantly by 2 h and remained elevated for the duration of the I/H infusion, the increase during S/G infusion was not significant until the final time point. The change in CBF was greater with I/H compared with S/G over the last 2 h of infusion.

Figure 1  Hormonal responses to the infusion of I/H (fat) and S/G (saline)

Values are means ± S.E.M. of 17 subjects. *P < 0.05 compared with baseline; †P < 0.05 compared with saline control day.

MSNA and vascular resistance

Changes in MSNA burst frequency and total MSNA in response to I/H and S/G infusions, along with CVR, are shown in Figure 2. MSNA recordings could not be obtained in four subjects because of shifts in electrode position or failure to meet signal-to-noise criteria. Furthermore, data for total MSNA were excluded if there was a shift in electrode position at any point during the experiment, leaving data from eight subjects for the total MSNA quantification. Owing to the challenges in maintaining the microneurography recording over a period of 4–5 h, we recruited more subjects than necessary to attain sufficient statistical power to identify differences...
Fatty acids stimulate sympathetic activity

Table 3  Baseline (0 h) and final (4 h) values for neural, cardiovascular and hormonal variables during infusion

<table>
<thead>
<tr>
<th>Variable</th>
<th>I/H</th>
<th>S/G</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>4 h</td>
<td>0 h</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>58.7 ± 2.4</td>
<td>69.0 ± 3.1*†</td>
<td>60.9 ± 2.3</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>109.2 ± 2.1</td>
<td>123.2 ± 2.7*†</td>
<td>112.4 ± 2.5</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>59.7 ± 1.9</td>
<td>68.5 ± 1.8*†</td>
<td>62.1 ± 1.4</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>76.2 ± 1.7</td>
<td>86.7 ± 2.0*†</td>
<td>78.8 ± 1.6</td>
</tr>
<tr>
<td>Q (fllres/min)</td>
<td>6.5 ± 0.4</td>
<td>6.4 ± 0.4</td>
<td>6.5 ± 0.4</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>86.7 ± 6.1</td>
<td>79.1 ± 6.9</td>
<td>85.3 ± 5.6</td>
</tr>
<tr>
<td>SVR (dyne · s · cm⁻¹)</td>
<td>991 ± 80</td>
<td>1151 ± 80*</td>
<td>1007 ± 56</td>
</tr>
<tr>
<td>MSNA (bursts/min)</td>
<td>16.3 ± 1.7</td>
<td>21.2 ± 1.8*</td>
<td>20.5 ± 2.2</td>
</tr>
<tr>
<td>Total MSNA (% baseline)</td>
<td>100 ± 0</td>
<td>165 ± 24*†</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>CBF (ml · 100 · m⁻¹ · min⁻¹)</td>
<td>1.65 ± 0.23</td>
<td>2.60 ± 0.29*†</td>
<td>1.95 ± 0.14</td>
</tr>
<tr>
<td>CVR (units)</td>
<td>54.4 ± 4.6</td>
<td>38.7 ± 3.2</td>
<td>45.1 ± 4.5</td>
</tr>
<tr>
<td>NA (nmol/l)</td>
<td>0.93 ± 0.06</td>
<td>0.74 ± 0.06*†</td>
<td>0.76 ± 0.07</td>
</tr>
<tr>
<td>Leptin (pg/ml)</td>
<td>5.43 ± 1.24</td>
<td>5.11 ± 1.16*</td>
<td>5.37 ± 1.19</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>2.35 ± 4.2</td>
<td>30.0 ± 4.3*†</td>
<td>23.3 ± 2.3</td>
</tr>
<tr>
<td>Adrenaline (pmol/l)</td>
<td>479.9 ± 34.0</td>
<td>491.6 ± 58.3</td>
<td>461.0 ± 35.3</td>
</tr>
<tr>
<td>Aldosterone (nmol/l)</td>
<td>0.08 ± 0.01</td>
<td>0.11 ± 0.01*†</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>AngII (pg/ml)</td>
<td>3.84 ± 0.58</td>
<td>3.02 ± 0.51</td>
<td>4.0 ± 0.62</td>
</tr>
<tr>
<td>F₂-isoprostanes (pg/ml)</td>
<td>12.4 ± 1.2</td>
<td>24.2 ± 1.1*†</td>
<td>12.9 ± 1.1</td>
</tr>
</tbody>
</table>

in MSNA in the case of missing data. Thus the more limited size of the MSNA sample should be regarded as adequate and representative of the overall sample. Of interest, haemodynamic responses were similar in individuals without and with suitable MSNA recordings.

The baseline burst frequency was not different between the two days, and Bland–Altman analysis revealed that 85% of the baseline MSNA values for both days were repeatable within 25%. This compares favourably with previous studies [21]. Both the change in burst frequency (+29.9% compared with +0.0%) and total MSNA (+65.1% compared with +12.3%) in comparison with baseline and S/G infusion were augmented after 2 h with I/H infusion and continued to increase until the end of infusion. CVR tended to decrease over the 4-h I/H infusion compared with S/G infusion (P = 0.08). Finally, the change in CVR during I/H infusion was directly related to the change in burst frequency (Figure 4).

**DISCUSSION**

The primary new findings of the present study were that (i) acute elevation of the plasma NEFA concentration with infusion of I/H increases central sympathetic activation measured from sympathetic post-ganglionic neurons, (ii) the augmentation of MSNA was associated with a rise in insulin, but not leptin, concentrations, and (iii) NEFAs increased aldosterone and F₂-isoprostanes, but not AngII, to contribute to the haemodynamic response.

Our present results confirm earlier reports that increased NEFA and/or TAG concentrations, as might be observed in insulin-resistant individuals following a meal, elevate BP and HR [7–9]. Our findings are similar to one study that reported a rise in SBP/DBP and HR by 13.5/8.0 2.1/1.5 mmHg and 9.4 1.4 beats/min respectively, following 4-h infusion of I/H, resulting from both an increase in SVR and Q [7]. However, the increase in BP in the present study resulted from augmented SVR, despite a rise in CBF and no change in Q. The observation that SVR was elevated while Q was unaltered and CBF was increased with lipid infusion suggests a redistribution of blood flow and vasoconstriction in vascular beds other than muscle [4]. Most studies are in agreement that NEFAs promote insulin resistance and impair endothelium-dependent vasodilation [22–24]; however, the endothelial dysfunction documented in these investigations is typically associated with a paradoxical increase in basal limb blood flow. Although few studies have examined the underlying mechanisms of increased basal limb blood flow from elevated NEFA levels [25–27], possible explanations include insulin-induced NO release or vasodilating prostaglandin production [26].

To date, limited findings suggest [6,8], but have not definitively proven [13,14], a link between the rise in BP and sympathetic activation. In normotensive rats,
the pressor response induced by oleic acid infusion was inhibited by the α1-adrenoceptor antagonist prazosin, suggesting sympathetic activation. Paolisso et al. [8] reported an increase in plasma NA with I/H infusion in humans, whereas another study [13] showed a small, but significant, decline in NA and a trend towards decreased renal venous NA spillover. It is difficult to reconcile these disparate findings, particularly in the absence of a control group and haemodynamic results in the latter study. More recently, a study assessing baroreflex function did not show a change in sympathetic activation during a 2-h lipid infusion [14]; however, the limited duration of that study may have precluded a complete sympathetic response to elevated NEFA concentrations. The increase in burst frequency (approx. 30 %) and total MSNA (approx. 65 %) recorded over the 4-h period in the present study clearly supports a role for central sympathetic activation in the pressor response to NEFAs. An additional finding in the present study is that the increase in SVR was directly associated with central sympathetic activation. Further studies blocking NEFA-induced sympathetic activation are necessary to determine a causal relationship.

Notably, NA concentrations decreased modestly during I/H infusion despite an increase in MSNA. This finding may at first appear counterintuitive; however,
several studies, including one with lipid infusion [13], have documented a dissociation of the MSNA–plasma NA relationship with a modulation of blood flow, neuronal re-uptake, NA degradation or NA clearance [28–32].

**Possible mechanisms of central sympathetic activation**

We considered four potential mechanisms by which NEFAs increased MSNA: (i) activation of vagal afferents, (ii) direct sensing in the hypothalamus, (iii) mediation by nutrient-sensing hormones, such as leptin and/or insulin, and (iv) modulation of the baroreflex. First, chemosensitive afferents arising from the abdominal viscera respond to lipids to influence discharge of vasomotor neurons of the rostral ventrolateral medulla [33]. Secondly, NEFAs cross the blood–brain barrier in proportion to circulating plasma NEFA concentrations and activate the POMC/CART (pro-opiomelanocortin/cocaine- and amphetamine-regulated transcript) neural pathway, resulting in decreased energy intake and activation of the SNS [34]. Although we cannot rule out the contribution of either of these mechanisms, the latter two can be examined in greater detail.

**Hormonal mediation**

Leptin and insulin have emerged as possible mediators of central sympathetic activation from NEFAs due to their characteristic responses to changes in adiposity and food intake. Both leptin and insulin stimulate POMC/CART neurons in the hypothalamus, which project to other brain centres and, ultimately, stimulate sympathetic pre-ganglionic neurons to modulate energy expenditure and BP [35]. Further support is shown by the observations that intravenous and intracerebroventricular administration of leptin increase both sympathetic activity and BP in conscious animals [36].

Our present results support a modest role for insulin as a mediator of sympathetic activation; however, leptin does not appear to be regulated extensively by elevated NEFA concentrations, at least in the acute setting. The approx. 30% increase in plasma insulin reported in the present study is in agreement with some [8,24,37], but not all [10,38], previous studies.

Previous studies have reported divergent findings regarding a leptin response to changes in NEFA concentrations. That peak leptin is positively correlated with a nocturnal peak in NEFA levels [39] suggests a link between NEFAs and leptin; however, results from lipid infusion in rats and humans are equivocal [40–43]. As the nadir for leptin levels occurs in the early afternoon, it is possible that the decline in leptin observed in the present study on the control day simply reflected the diurnal rhythm, whereas elevated NEFA levels supported maintenance of leptin concentration. Nevertheless, its null response to NEFAs precludes leptin as a sympathetic activator in this study.

**Baroreflex modulation**

Two studies have presented conflicting evidence regarding acute baroreflex resetting and reduced baroreflex sensitivity in response to 1-h lipid infusion [14,44]. Although our present study was not designed to assess baroreflex function, the concomitant increase in BP, HR and MSNA supports the concept of resetting of the arterial baroreflexes to defend higher pressures. Alternatively, increases in MSNA may be a reflex response to muscle vasodilation with lipid infusion; however, two observations refute this notion. First, the BP response to I/H infusion far exceeds the expected baroreflex compensation, although CBF increased 20% within 1 h and 60% by 4 h of I/H infusion. The expected final MAP if the increase in CBF is representative of all skeletal muscle and if Q and resistance in non-skeletal muscle beds remained at control levels would be 72.8 mmHg compared with the basal MAP of 76.2 mmHg, considerably less than the measured BP after I/H infusion. Secondly, as MSNA did not increase until after 2 h of infusion with I/H, whereas CBF and BP increased by 1 h, it is unlikely that MSNA augmentation resulted from a pure baroreflex response.

**Could peripheral mechanisms contribute to the pressor response?**

Although central sympathetic activation contributes to the pressor response to NEFAs, results from the present study also suggest other peripheral mechanisms are involved. As in previous studies [9], F2-isoprostanes, a biomarker of oxidative stress that has been linked to hypertension, doubled during I/H infusion in the present study, supporting its role in the BP response to NEFAs. Although no changes were observed in AngII concentrations on either experimental day, aldosterone increased approx. 38% by the end of I/H infusion, in contrast with the approx. 44% fall on the control day. The increase in aldosterone is consistent with a previous study that demonstrated the adrenal-stimulating effects of oxidized linoleic acid derivatives [45]. The reason for the decline in aldosterone on the control day is unclear; however, it is likely that the postural shift in blood volume and central venous pressure is a controlling factor [46]. Other mediators of a pressor response to NEFAs not measured in the present study include direct stimulation of protein kinases that cause constriction and increased sensitization of vessels to α-adrenergic stimulation [19]. From our present findings it appears likely that both peripheral and central mechanisms play a role in the haemodynamic response to elevated NEFA concentrations. Interestingly, in agreement with a previous study [7], a biphasic response was observed such that BP increased within the first 30 min and remained relatively stable,
followed by an even greater increase during the final 2 h of lipid infusion. Additionally, in our present study, HR and SVR did not begin to rise until the final 2 h, coinciding with the increase in MSNA. These observations may reflect an initial direct vascular effect of fat followed by central activation (after approx. 2 h of infusion) and the corresponding haemodynamic responses.

**Limitations**

Results from previous studies have suggested that an infusion duration of at least 3–4 h was necessary to evoke the hypothesized hormonal, neural and haemodynamic responses [8,43]. Owing to the technical challenges of maintaining sympathetic nerve recordings, particularly due to the length of the study, two issues were of concern. First, any leg movement by the subject could alter the nerve recording or disrupt the signal altogether. Consequently, results could not be analysed for burst frequency in approx. 20 % and for total MSNA in approx. 50 % of subjects. Despite the reduction in MSNA data, the statistical power was large enough to identify differences for lipid infusion compared with control. Secondly, it is possible that stress or bladder distension contributed to the sympathetic and haemodynamic response [47] over the approximate 5-h basal and infusion period. To mitigate bladder distension, subjects voided using a condom catheter or urinal while remaining supine and stationary. Finally, the findings result from an acute period. To mitigate bladder distension, subjects voided using a condom catheter or urinal while remaining supine and stationary. Finally, the findings result from an acute

**Conclusions**

The present study provides direct evidence that in humans the pressor response to NEFAs is mediated, at least in part, by sympathetic activation. Moreover, insulin, F2-isoprostanes and aldosterone, but not leptin, may contribute to the haemodynamic response during lipid infusion. The neurogenic response documented in the present study implicates central sympathetic activation as a possible mechanism linking conditions characterized by high NEFA concentrations (e.g. obesity and dyslipidaemia) with hypertension. With the age-adjusted prevalence rate of hypertension in the U.S.A. now over 28 % [48], due in large part to the increasing obesity epidemic, more investigations of the mechanistic underpinnings of the NEFA–SNS link are warranted. In order to more precisely elucidate the relationship between NEFAs and sympathetic activation in some forms of hypertension, future studies should examine the effect of more chronic elevations of NEFA levels on autonomic control in humans and whether reducing the NEFA concentration results in a corresponding decrease in sympathetic activity.

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