Effect of acute hyperlipidemia on autonomic and cardiovascular control in humans

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Monahan KD, Dyckman DJ, Ray CA. Effect of acute hyperlipidemia on autonomic and cardiovascular control in humans. J Appl Physiol 103: 162–169, 2007. First published April 19, 2007; doi:10.1152/japplphysiol.00167.2007.— Blood lipids may detrimentally affect autonomic and circulatory control. We tested the hypotheses that acute elevations in free fatty acids and triglycerides (acute hyperlipidemia) impair baroreflex control of cardiac period [cardiovascular baroreflex sensitivity (BRS)] and muscle sympathetic nerve activity (MSNA: sympathetic BRS), increase MSNA at rest, and augment physiological responses to exercise. Eighteen young adults were examined in this randomized, double-blinded, and placebo-controlled study. BRS was determined using the modified Oxford technique before (pre) and 60 min (post) after initiating infusion of Intralipid (0.8 ml·m⁻²·min⁻¹) and heparin (1,000 U/h) (experimental; n = 12) to induce acute hyperlipidemia, or saline (0.8 ml·m⁻²·min⁻¹) and heparin (1,000 U/h) (control; n = 6). Responses to isometric handgrip to fatigue (IHG) were also determined. Blood pressure increased more (P < 0.05) in experimental than control subjects during the infusion. MSNA at rest (14 ± 2 vs. 11 ± 1 bursts/min), cardiovascular (19.8 ± 1.8 vs. 19.1 ± 2.4 ms/mmHg pre and post, respectively) and sympathetic BRS (−5.5 ± 0.6 vs. −5.2 ± 0.4 au·beat⁻¹·mmHg⁻¹), and the neural and cardiovascular responses to IHG were unchanged by acute hyperlipidemia (pre vs. post) in experimental subjects. Similarly, MSNA at rest (10 ± 2 vs. 12 ± 2 bursts/min), cardiovascular (22.1 ± 4.0 vs. 21.0 ± 4.6 ms/mmHg) and sympathetic BRS (−5.8 ± 0.5 vs. −5.5 ± 0.5 au·beat⁻¹·mmHg⁻¹), and the neural and cardiovascular responses to IHG were unchanged by the infusion in control subjects. These data do not provide experimental support for the concept that acute hyperlipidemia impairs reflex cardiovascular or sympathetic regulation in humans.

autonomic nervous system; baroreceptor; blood pressure; fatty acid; obesity; visceral adiposity

ACUTE INFUSION OF THE FREE FATTY ACID (FFA) oleate into the portal vein of rats mediates a pressor response (13), which appears to be sympathetically mediated (11). Since abdominal obesity is associated with enhanced FFA release and turnover (23), it is possible that FFAs may contribute to or explain the association between increased abdominal adiposity and hypertension (30). Furthermore, these data are consistent with experimental evidence that the sympathoexcitation associated with obesity, as measured by increased muscle sympathetic nerve activity (MSNA) (10), may be mediated by increased abdominal (i.e., visceral) rather than subcutaneous fat (1, 2). In previous studies, plasma norepinephrine (a gross index of sympathetic outflow) measured before and after acutely inducing hyperlipidemia has been shown to increase (13, 22, 29), decrease (21), and be unchanged (11). Additionally, in humans, a single uncontrolled study reported that renal and whole body norepinephrine spillover tended to decrease rather than increase during acute hyperlipidemia (12). Thus, to date, the scientific evidence to support a sympathoexcitatory effect of hyperlipidemia has not consistently been shown. These inconsistencies may arise in part due to the indirect nature of the measure used to quantify sympathetic outflow.

In addition to the possibility that acute hyperlipidemia elicits sympathoexcitatory effects, previous studies in humans have observed impaired cardiac baroreflex function after acute hyperlipidemia (8, 9). Impaired baroreflex function may be important in several different regards. First, impaired cardiac baroreflex function is associated with increased risk of sudden cardiac death in some populations (20), and this association may help explain the link between increased FFA concentrations and sudden cardiac death (17). Second, baroreflex dysfunction may contribute to increased sympathetic outflow at rest due to an impaired ability of the baroreflexes to tonically restrain sympathetic outflow. Although these previous studies (8, 9) are important, they do not provide evidence that systematic changes in baroreflex function occurred since only the cardiac arm, and not the sympathetic arm of the baroreflex, was studied.

Accordingly, in the present study, we tested the hypotheses that acute hyperlipidemia impairs baroreflex control of cardiac period and sympathetic outflow and increases sympathetic outflow at rest. Additionally, if baroreflex dysfunction occurred, we wanted to determine whether this was associated with functional changes in the neural and cardiovascular responses to physiological stress (exercise).

METHODS

Subjects

We studied 18 young (18–35 yr old), healthy, normotensive [blood pressure (BP) of <140/90 mmHg], nonsmoking, and nonobese (body mass index of <30 kg/m²) subjects. The study was reviewed and approved by the Pennsylvania State University College of Medicine Institutional Review Board, and written, informed consent was obtained from all subjects before testing on approved forms.

Measurements

Subjects were studied supine in the fasted state (12 h). BP and heart rate. Resting BP was determined with a Dinamap monitor. Continuous BP was obtained using a Finapres (Ohmeda) monitor. ECG was used to determine heart rate.

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MSNA. Direct intraneural multifiber recordings of MSNA were obtained from the peroneal nerve using microneurography (37). Raw nerve recordings were transformed to mean voltage neurograms by amplification, filtering (700–2,000 Hz), full-wave rectification, and integration (0.1-s time constant).

Cardiovagal and sympathetic baroreflex sensitivity. Cardiovagal and sympathetic baroreflex sensitivity (BRS) was determined using the modified Oxford technique (6, 32). This method involves sequential bolus intravenous infusion of nitroprusside (75 μg) followed 60 s later by phenylephrine (125 μg). Dosages of nitroprusside and/or phenylephrine were increased by 25 μg in subsequent trials (performed at least 20 min later) if the desired effect on BP was not obtained (~15-mmHg decrease and increase in BP from resting levels) (27) (Fig. 1). Data acquisition began 3 min before (Pre) and continued for 3 min after (Post) nitroprusside infusion. Drug doses were identical within a given subject Pre and Post.

Isometric handgrip to fatigue. Maximal voluntary contraction force of the left forearm was determined in triplicate before instrumenting the subject for the protocol. During the isometric handgrip (IHG) trial, handgrip was performed at 30% of maximal voluntary contraction until target force could no longer be maintained.

Blood samples. Venous blood samples were obtained at baseline (before making Pre measurements) and at ~60 and ~120 min after beginning the infusion (see below). Total, high-density, and low-density cholesterol as well as triglycerides were quantified from plasma samples collected in lithium-heparinized tubes using an Olympus AU640 chemistry analyzer. Serum for FFA samples were collected and frozen (serum) until being analyzed using a Wako Diagnostics enzymatic assay (NEFA-C).

Fig. 1. Representative data from a baroreflex trial. Top: continuous beat-by-beat recording of the systolic BP (solid line; top), diastolic BP (broken line; top), R-R interval (middle), and muscle sympathetic nerve activity (MSNA) response (bottom). Nitroprusside bolus infusion occurs at minute 0 followed at minute 1 by a phenylephrine. These infusions decrease then increase BP, eliciting baroreflex-mediated changes in R-R interval and MSNA. Bottom: regressions between R-R interval and systolic BP (cardiovagal baroreflex sensitivity [BRS]; left) and MSNA and diastolic BP (sympathetic BRS; right) during changes in BP.
Experimental Protocol

Two BRS trials (Pre 1 and Pre 2) and an IHG to fatigue trial were performed at baseline. After obtaining these baseline (Pre) measurements, subjects received an intravenous infusion of either saline (0.8 ml·m⁻²·min⁻¹) with heparin (1,000 U/h after a 200-U bolus) (control) or a fat emulsion (Intralipid 20%; 0.8 ml·m⁻²·min⁻¹) with heparin (1,000 U/h after a 200-U bolus) (experimental). This corresponded to an absolute fluid infusion rate of 103 ± 3 and 97 ± 3 ml/h in control and experimental subjects, respectively. Heparin was infused in experimental subjects to activate lipoprotein lipase activity and facilitate turnover of triglycerides to FFAs. To account for any nonspecific effect of heparin, control subjects also received heparin in their infusion. Assignment to the control or experimental group occurred randomly in a 2:1 fashion (2 subjects randomized to the control group for every 1 randomized to the experimental group) since their infusion. Assignment to the control or experimental group occurred randomly in a 2:1 fashion (2 subjects randomized to the experimental group for every 1 randomized to the control group) since our laboratory recently established the stability of all these measures in subjects not exposed to any experimental intervention (i.e., time control subjects) (25–28). Subjects were unaware to which group they were assigned. Only the nurse had knowledge of the infusates. During the second hour of the continuous infusion, the BRS trials (Post 1 and Post 2) and IHG trial were repeated (Fig. 2). BP and heart rate at rest were determined in triplicate before each trial (Pre and Post). MSNA at rest was determined during data collected in the 3-min baseline period before each trial. Each trial was separated by at least 20 min to avoid residual effects from the previous trial. The IHG trial was always performed after the BRS trials.

Data Analysis

Physiological data were digitally recorded (MacLab 8e, AD Instruments) at 400 Hz. MSNA at rest was quantified as bursts per minute, bursts per 100 heartbeats, and sum of the area under individual bursts per minute (au/min). Neurograms were calibrated at rest by assigning the tallest burst during the respective baseline period an amplitude of 1,000 and setting the baseline to 0 (period in which no sympathetic bursts occurred for >3 consecutive cardiac cycles).

Cardiovagal BRS is reported as the linear slope of the R-R interval-systolic BP relation (averaged over 2-mmHg pressure ranges) from the nadir (post-nitroprusside bolus) to peak (post-phenylephrine) systolic BP response during each trial (6, 32). Sympathetic BRS is reported as the linear slope of the MSNA-diastolic BP relation (averaged over 3-mmHg pressure ranges) from the beginning of the nitroprusside-induced decrease to the peak post-phenylephrine bolus diastolic BP response during each trial (6, 32). Points clearly contained in either the threshold or saturation region of the stimulus-response curve were visually identified and manually removed (16). MSNA was quantified as the area underlying sympathetic bursts. After the neurograms were shifted to account for conduction velocity delays (~1.2–1.4 s), sympathetic bursts were visually identified.

Heart beats not associated with sympathetic bursts were assigned a value of zero and were included in the analyses over each diastolic BP range (6, 32). Diastolic BP ranges with no sympathetic activity were excluded from the analyses (32). Cardiovagal BRS trials with r values of ≥0.70 (32, 33) were averaged, and a single mean value is reported for both Pre and Post. All trials in the present study met these criteria (r ≥ 0.70). A similar process occurred for sympathetic BRS, except the criteria for retaining a trial was an r value of ≥0.50 (4, 32). Four trials did not meet this criterion (r ≤ 0.50). Three in control subjects (single Pre 2, single Post 1, single Post 2 trials) and one from an experimental subject (Pre 2 trial). An investigator blinded to the experimental condition performed all analyses.

Responses to IHG were quantified over 30-s intervals. Responses to IHG were made at the same relative time points (i.e., at 25%, 50%, 75%, and 100% of handgrip duration) to allow comparisons at comparable physiological time points within subjects (Pre vs. Post) and between groups (control vs. experimental).

Statistical Analysis

Differences in subject characteristics at baseline were determined by unpaired t-test. Responses to the intervention were determined using a repeated-measures ANOVA. Statistical significance occurred when P < 0.05.

RESULTS

Subject Characteristics

Subjects in the experimental and control groups were well matched at baseline (Table 1). In response to the respective infusions, triglycerides and FFAs increased in the experimental group (Table 2). Additionally, systolic, diastolic, and mean BP increased (P < 0.05) in both experimental and control subjects (Table 2). However, increases were more pronounced in experimental subjects (group-by-time interaction, P < 0.05).
The neural (MSNA) and cardiovascular (BP and heart rate) responses to IHG to fatigue are presented in Fig. 5. Responses to IHG to fatigue are presented in Fig. 5. Responses were similar in both the experimental and control groups before beginning the infusion (Pre) and were unchanged by infusion (Post) in either group (Fig. 5). Time to fatigue decreased in both control (253 ± 29 vs. 197 ± 22 s for Pre and Post, respectively; \( P < 0.05 \)) and experimental groups (287 ± 26 vs. 243 ± 30 s; \( P < 0.01 \)).

### DISCUSSION

The novel experimental findings provided from this randomized, double-blinded, and placebo-controlled study are that acute hyperlipidemia 1) does not impair the ability of the baroreflexes to respond to acute perturbations in systemic BP by altering cardiac period (cardiovagal BRS) or MSNA (sympathetic BRS), 2) tended to decrease sympathetic outflow as measured directly using microneurography (MSNA) at rest, and 3) does not alter the pressor or neural responses to a physiological stressor (IHG). Importantly, the magnitude of the hyperlipidemia induced was at least as great as, and likely greater than, that seen chronically in disease (5, 14) or acutely after ingestion of a high-fat meal (35, 38). Thus the lack of effect of acute hyperlipidemia on autonomic control likely cannot be explained by an insufficiency stimulus.

### Table 2. Response to the intervention

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre</th>
<th>Post</th>
<th>Post 1</th>
<th>Post 2</th>
<th>Post 3</th>
</tr>
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<tbody>
<tr>
<td>Systolic BP, mmHg</td>
<td>116.2±5</td>
<td>116±6</td>
<td>118.5±5</td>
<td>120±5*</td>
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<tr>
<td>Diastolic BP, mmHg</td>
<td>72.3±3</td>
<td>70±3</td>
<td>73±2</td>
<td>73±4*</td>
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<tr>
<td>Mean BP, mmHg</td>
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<td>85.5±4</td>
<td>88.3</td>
<td>89.4±4</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>56±2</td>
<td>60±3</td>
<td>56±3</td>
<td>59±2</td>
<td></td>
</tr>
<tr>
<td>MSNA, bursts/min</td>
<td>10±2</td>
<td>10±2</td>
<td>10±2</td>
<td>12±2</td>
<td></td>
</tr>
<tr>
<td>MSNA, bursts/100 beats</td>
<td>19±2</td>
<td>17±2</td>
<td>18±4</td>
<td>21±3</td>
<td></td>
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<tr>
<td>Triglycerides, mmM</td>
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<td>0.95±0.19</td>
<td></td>
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<td>Free fatty acids, µM</td>
<td>0.44±0.09</td>
<td>0.84±0.27</td>
<td></td>
<td>0.80±0.22</td>
<td></td>
</tr>
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</table>

Values are means ± SE. Pre (baseline) measurements were obtained before beginning the intervention (infusion). Post measurements were obtained –60 (Post 1), –90 (Post 2), and –120 min (Post 3) after beginning infusion of saline and heparin (control subjects) or Intralipid and heparin (experimental subjects). During these Post measurements, the infusion continued, BP, blood pressure; MSNA, muscle sympathetic nerve activity. * \( P < 0.05 \) main effect (intervention). † \( P < 0.05 \) interaction (group × intervention).

Heart rate increased slightly in experimental (\( P < 0.05 \)) but not control subjects (Table 2). MSNA at rest expressed in units of bursts per minute tended to decrease (\( P = 0.09; \) Table 2) over time in experimental subjects. In contrast, when MSNA was expressed as bursts per 100 heartbeats, this effect was significant (\( P < 0.05; \) Table 2). In control subjects, MSNA (bursts/min and bursts/100 heartbeats) did not change over the course of the study (Table 2).

### Cardiovagal and Sympathetic BRS

Cardiovagal and sympathetic BRS were measured in all subjects (experimental, \( n = 12 \) and control subjects, \( n = 6 \)) before (Pre) and during (Post) infusion. At baseline (Pre), cardiovagal and sympathetic BRS were similar in the experimental and control groups (Fig. 3). Cardiovagal (19.8 ± 1.8 vs. 19.1 ± 2.4 ms/mmHg for Pre and Post, respectively; Fig. 3) and sympathetic BRS (–5.5 ± 0.6 vs. –5.2 ± 0.4 au·beat⁻¹·mmHg⁻¹; Fig. 3) were unchanged by Intralipid-heparin infusion in the experimental subjects. Similarly, cardiovagal (22.1 ± 4.0 vs. 21.0 ± 4.6 ms/mmHg; Fig. 3) and sympathetic BRS (–5.1 ± 0.8 vs. –5.5 ± 0.8 au·beat⁻¹·mmHg⁻¹; Fig. 3) were unchanged by saline-heparin infusion in the control group.

### Neural and Cardiovascular Responses to IHG to Fatigue

The neural (MSNA) and cardiovascular (BP and heart rate) responses to IHG to fatigue are presented in Fig. 5. Responses were similar in both the experimental and control groups before beginning the infusion (Pre) and were unchanged by infusion (Post) in either group (Fig. 5). Time to fatigue decreased in both control (253 ± 29 vs. 197 ± 22 s for Pre and Post, respectively; \( P < 0.05 \)) and experimental groups (287 ± 26 vs. 243 ± 30 s; \( P < 0.01 \)).

**Fig. 3.** Cardiovagal and sympathetic baroreflex sensitivity (BRS) before (Pre) and during (Post) infusion of heparin (control: open bars) or Intralipid with heparin (experimental: filled bars). All values are means ± SE.
In contrast to our finding of preserved cardiovagal BRS in the face of acute hyperlipidemia, two previous human studies reported impairments using an identical infusion protocol (8, 9). There are at least several differences between these previous studies and ours that may help to explain the discrepant findings. First, the prior studies (8, 9) did not include appropriate experimentally indicated control groups. This critical exclusion makes it impossible to determine whether the observed detrimental effect of acute hyperlipidemia on baroreflex function was the effect of the Intralipid-heparin infusion per se. The stability we demonstrate across time in our control group does not provide any level of assurance that similar effects would have been observed by other investigators had a time-control group been included (8, 9). Second, the prior studies measured baroreflex function during either prolonged (59 min) steady-state infusions of phenylephrine (8) or in response to phenylephrine boluses administered 1–2 min apart (9). It is likely that steady-state infusions of this type result in baroreflex-mediated bradycardia, which involve both parasympathetic and sympathetic components (19). This likely explains why results from baroreflex trials using steady-state infusions and bolus infusions of vasoactive drugs to assess baroreflex function do not correlate (36). Thus our cardiovagal BRS responses may not be directly comparable to those of Gadegbeku et al. (8). In regard to baroreflex data being obtained by infusing phenylephrine boluses at 1- to 2-min intervals, it is clear that residual effects of the prior trial are likely to still be manifest (Fig. 1; note significant elevation in BP still apparent 2 min after phenylephrine bolus). The role that this exerts on the ability of the baroreflex to respond to subsequent trials occurring at that time is unknown. Gadegbeku et al. did not provide any data illustrating the reproducibility of the data derived using this measure (9), and we are aware of no such data in the literature. This comment serves to further illustrate why a control group was essential in these prior studies. Third, baroreflex responses were measured only during increases in BP (8, 9), which may have limited the portion of the stimulus-response curve that was revealed. Our study more comprehensively examined the reflex response both above and below resting BP levels in an attempt to capture a greater portion of the linear stimulus-response curve. Additionally, we removed portions of the stimulus-response clearly contained in the threshold and saturation regions. From the descriptions in previous studies, it does not appear that similar attempts were made (8, 9). Thus it is possible that data from the nonlinear portions of the stimulus-response curve may have been retained in the analysis. Collectively, these issues may help to explain the differences observed between past studies (8, 9) and our present study.

In addition to our demonstration of unaltered cardiovagal BRS during acute hyperlipidemia, we observed no effect on sympathetic BRS. This study is the first to determine this effect. This finding is important to document since it cannot be assumed that a given effect of an intervention on either cardiovagal or sympathetic BRS indicates global changes in baroreflex function (7, 24). Thus, collectively, the results of our rigorously designed study strongly suggest that acute hyperlipidemia does not impair the ability of the baroreflexes to respond to

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**Fig. 4.** Individual responses in cardiovagal (top) and sympathetic baroreflex sensitivity (BRS) (bottom) before (Pre) and during (Post) infusion of heparin (control; left) or Intralipid with heparin (experimental; right). Mean responses are denoted by closed symbols and individuals as open symbols.
baroreceptor activation/deactivation by altering either cardiac period (cardiovagal BRS) or MSNA (sympathetic BRS).

Previous animal studies suggest that the pressor response to increased FFAs is sympathetically mediated (11, 13). This effect could result from increased sympathetic outflow or increased end organ responsiveness to sympathetic stimuli. In the present study, we did observe a modest pressor effect at rest during acute hyperlipidemia. However, these increases were

Fig. 5. Responses to IHG before (Pre) and ~120 min after beginning infusion of heparin (control; n = 6) or Intralipid with heparin (experimental; n = 12). Data are plotted as changes from resting baseline (BL) and times corresponding to 25, 50, 75, and 100% of fatigue. Responses to IHG were very similar before (Pre) and during (Post) infusion in both control and experimental subjects.
not associated with increased MSNA. In fact, if anything, MSNA tended to decrease when expressed as bursts per minute \((P = 0.09)\) and did decrease when expressed as bursts per 100 heartbeats during acute hyperlipidemia. These data are consistent with a previous study in humans that showed that acute hyperlipidemia tended to decrease whole body and renal norepinephrine spillover with no effect on forearm norepinephrine spillover being observed \((31)\). Thus, taken collectively, the pressor responses to acute FFA elevations in humans is unlikely to be mediated by increased sympathetic outflow. Possible explanations for the observed blood lipid-induced pressor response should include the possibility that a blunted dilator signal rather than an enhanced constrictor signal may underlie such increases. For instance, FFAs may elicit a pressor effect by impairing endothelial function \((35)\) or increasing vasoconstrictor byproducts of arachidonic acid metabolism \((15)\).

Several limitations may be associated with the present study. First, the duration of the infusion in the present study was short \((-2 \text{ h})\). We cannot exclude the possibility that different results would have been obtained had a longer duration infusion been used. However, it is difficult to increase the duration of studies in which intraneural recordings need to be maintained. Second, the present results likely provide more insight into the effect of acute, such as occurs after ingestion of a high fat meal, rather than chronic hyperlipidemia associated with some forms of obesity on autonomic control. Third, sympathetic outflow, although directly measured, included only efferent sympathetic outflow directed toward skeletal muscle. Thus we cannot extrapolate these findings to other organs. Fourth, our subjects were young and healthy. It is possible that different effects of acute hyperlipidemia may be observed in other populations such as obese or dyslipidemic subjects. Fifth, some \((29, 34)\) but not all \((12, 18)\) studies using a similar experimental protocol to increase FFA levels have shown that insulin levels increase. We did not measure insulin levels in the present study. Therefore, we cannot determine whether changes in circulating insulin influenced our results. However, if they did increase, they are likely to have obscured the true magnitude of our observed effects (pressor effect and sympathoinhibitory effect) since hyperinsulinemia in young healthy adults increases MSNA and produces peripheral vasodilation without affecting BP at rest \((3)\).

In conclusion, the present findings indicate that acute hyperlipidemia does not impair the ability of the baroreflexes to respond to perturbations in systemic BP by altering cardiac period (cardiovagal BRS) or MSNA (sympathetic BRS) and does not modify neural and pressor responses to physiological stress (IHG), and that lipid-induced pressor responses are not associated with increased MSNA.

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