Acute haemodynamic effects of lipolysis-induced increase of free fatty acids in healthy men

Mark T. KEARNEY*, Philip J. CHOWIENCZYK†, Sally E. BRETT†, Angela SUTCLIFFE*, James M. RITTER† and Ajay M. SHAH*

*Department of Cardiology, GKT School of Medicine, Denmark Hill, Bessemer Road, London SE5 9PJ, U.K., and †Clinical Pharmacology, St Thomas’ Hospital, Lambeth Palace Rd, London SE1 7EH, U.K.

ABSTRACT

Circulating free fatty acids (FFA) are elevated in subjects with insulin resistance and Type II diabetes, and increase during myocardial ischaemia, but their haemodynamic effects are incompletely understood. During an investigation of the effects of FFA on endothelial function, we administered lipid emulsion (150 mg min\(^{-1}\) of soybean oil) with heparin (0.2 unit kg\(^{-1}\) min\(^{-1}\)) intravenously to eight healthy men for 2 h. This increased circulating FFA to 3.1 ± 0.5 mmol/l. Forearm blood flow was measured by venous occlusion plethysmography during brachial artery infusions of saline, acetylcholine and nitroprusside before, and at 1 and 2 h. Lipid/heparin infusion had no significant effect on vasodilation to nitroprusside but progressively increased responses to acetylcholine (from 6.3 ± 2.0 during 30 μg min\(^{-1}\) before-lipid infusion to 7.9 ± 1.3 at 1 h and 12.2 ± 1.1 ml min\(^{-1}\) 100 ml\(^{-1}\) at 2 h, \(P < 0.001\)). Basal flow increased from 2.7 ± 0.7 to 4.7 ± 0.8 ml min\(^{-1}\) 100 ml\(^{-1}\) from 0 to 2 h. We performed a second study to clarify this effect on basal blood flow. Healthy men (n = 8) received, on separate occasions, 4 h intravenous infusions of lipid emulsion with heparin and, as a control, saline with heparin. Lipid with heparin increased mean arterial blood pressure (maximum increment 8.2 ± 2.7 mmHg, \(P < 0.01\) compared with saline/heparin control) and forearm blood flow (from 1.7 ± 0.2 to 2.9 ± 0.3 ml min\(^{-1}\) 100 ml\(^{-1}\), \(P < 0.01\) without a significant effect on heart rate, and reduced calculated forearm vascular resistance (from 49.1 ± 5.4 to 31.3 ± 3.9 arbitrary units, \(P < 0.01\)). In conclusion, acute elevation of FFA in healthy men increases arterial blood pressure and reduces vascular resistance. These haemodynamic changes could be clinically relevant.

INTRODUCTION

Endothelial dysfunction, thought to be an early event in atherogenesis [1], is particularly marked in subjects with Type II diabetes [2] and insulin-resistant non-diabetic subjects [3], conditions characterized by an elevation of plasma free fatty acids (FFA) [4]. The effect of FFA on endothelial function has received much attention but remains controversial [5–10]. We therefore examined forearm vasodilator responses to brachial artery infusions of an endothelium-dependent and an endothelium-independent vasodilator before and during intravenous infusion of a lipid emulsion with heparin. This infusion increased circulating FFA to concentrations observed during acute myocardial ischaemia [11]. An important finding was an increased basal forearm blood flow during lipid infusion. We therefore explored the effect of elevating circulating FFA on blood pressure and forearm blood flow in a separate study.

Key words: free fatty acid, blood pressure, heart rate, forearm blood flow, acetylcholine.

Abbreviations: FFA, free fatty acids; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Correspondence: Dr Mark T. Kearney (e-mail mark.kearney@kcl.ac.uk).
METHODS

Subjects

Studies were performed in healthy normotensive male non-smokers. No subject had a family history of diabetes mellitus. The St Thomas’ Hospital research ethics committee approved the studies and all subjects gave informed written consent. The investigation conformed to the principles outlined in the Declaration of Helsinki.

Protocols

Two protocols were performed in separate groups of healthy men. Study I (n = 8) tested endothelial function during a 2 h intravenous infusion of lipid emulsion. Forearm blood flow during brachial artery infusion of saline, acetylcholine and sodium nitroprusside was measured at baseline and at 1 and 2 h. Study II employed a crossover design in which each subject received lipid and control infusions on separate occasions one week or more apart; brachial artery infusions were not performed and lipid was infused for 4 h but the protocol was otherwise similar to Study I. Subjects attended the clinical investigation unit at 8 am after an overnight fast. They lay supine in a temperature controlled room (24 ± 2 °C). Heparin (100 units/ml; Antigen, Republic of Ireland) and Intralipid™ [20% (v/v) oil in water emulsion containing purified soybean oil, fractionated egg phospholipids, glycerol and water (Pharmacia Inc.)] or the same volume of normal saline/heparin were infused into an antecubital vein. Lipid emulsion was infused at a rate of 45 ml·h⁻¹ (corresponding to a dose of soybean oil of 150 mg·min⁻¹). Heparin 500-units bolus followed by 0.2 unit·kg⁻¹·min⁻¹ was used to activate lipoprotein lipase and hence increase plasma FFA [12].

Haemodynamic measurements

Forearm blood flow was measured using venous occlusion plethysmography with electrically calibrated strain gauges [13–15]. Collecting cuff pressure was 40 mmHg and wrist cuff occlusion pressure 180 mmHg. Heart rate and arterial blood pressure were measured using an Omron HEM 705C blood pressure monitor. Endothelial function (Study I) was determined by measuring changes in forearm blood flow in response to brachial artery infusion of acetylcholine (endothelium-dependent) and nitroprusside (endothelium-independent) vasodilators dissolved in saline. The brachial artery of the left arm was cannulated with a 27-standard wire gauge steel needle (Cooper’s Needle Works, Birmingham, U.K.) under 1% (w/v) lignocaine (Xylocaine, Astra Pharmaceutical, Kings Langley, U.K.) local anaesthesia, and attached to a 16-gauge epidural catheter (Portex, Hythe, U.K.). Patency was maintained by infusion of saline via an IVAC P1000 syringe pump (IVAC, Basingstoke, U.K.). The total rate of intravenous arterial infusions was maintained constant throughout all studies at 1 ml·min⁻¹. Subjects rested supine for 15 min after needle placement before blood flow measurements. Cumulative infusions of acetylcholine chloride (7.5, 15 and 30 µg·min⁻¹; Giba Vision Ophthalmics, Southampton, Hants., U.K.) and sodium nitroprusside (1, 5 and 10 µg·min⁻¹; David Bull Laboratories, Victoria, Australia) were then carried out. Each dose was infused for 5 min and the infusion of each drug was separated by normal saline for 15 min. Forearm blood flow was recorded during the last 3 min of each infusion period. Means of the final five measurements were used for analysis.

Plasma assays

Blood (20 ml) samples were collected from a cannula in a dorsal hand vein into EDTA and plain tubes. Plasma was separated immediately after collection by centrifugation at 2200 g for 15 min at 4 °C. Plasma was then frozen and stored at −80 °C for up to 6 weeks before analysis. Plasma glucose concentration was measured with a Beckman Glucose analyser II (Beckman Instruments, Fullerton, CA, U.S.A.) using the glucose oxidase method. Insulin concentrations were determined by a double antibody radioimmunoassay (Pharmacia Insulin RIA kit; Pharmacia Upjohn, Milton Keynes, U.K.) after precipitation with polyethylene glycol. Triacylglycerol, cholesterol and total fatty acid concentrations were determined enzymically (Wako Chemicals, Richmond, VA, U.S.A.).

Calculations and data analysis

Results are summarized as means ± S.E.M. Mean arterial blood pressure is expressed as mmHg and was calculated using the expression DBP + (SBP – DBP) / 3, where DBP and SBP are diastolic and systolic blood pressure respectively. Forearm blood flow is expressed in ml·min⁻¹·100 ml⁻¹. Forearm vascular resistance (expressed in arbitrary units) was calculated from mean blood pressure divided by blood flow. Blood pressure and forearm blood flow responses are expressed as change from the immediately preceding baseline. Repeated measures ANOVA was used to compare changes in metabolic and haemodynamic measurements in response to agonists and FFA. Where ANOVA showed a significant difference, post-hoc t tests were carried out with application of a Bonferroni correction. Statistical significance was accepted at a level of P < 0.05.

RESULTS

Baseline fasting metabolic and haemodynamic data for both studies are shown in Table 1.
Table 1  Baseline characteristics of subjects

<table>
<thead>
<tr>
<th></th>
<th>Study I</th>
<th>Study II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Age (years)</td>
<td>29 ± 1</td>
<td>34 ± 1</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.2 ± 0.7</td>
<td>25.6 ± 0.8</td>
</tr>
<tr>
<td>Fasting cholesterol (mmol/l)</td>
<td>4.2 ± 0.3</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td>Fasting LDL (mmol/l)</td>
<td>2.2 ± 0.3</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Fasting HDL (mmol/l)</td>
<td>1.4 ± 0.5</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>Fasting triglycerol (mmol/l)</td>
<td>1.3 ± 0.3</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>Fasting FFA (mmol/l)</td>
<td>0.52 ± 0.07</td>
<td>0.59 ± 0.70</td>
</tr>
<tr>
<td>Fasting insulin (m-units/l)</td>
<td>4.8 ± 0.6</td>
<td>5.3 ± 1.1</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>4.3 ± 0.1</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>76.0 ± 2.0</td>
<td>84.5 ± 3.8</td>
</tr>
</tbody>
</table>

**Study I**

**Metabolic data**

Glucose, insulin, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) did not change significantly during the lipid infusion. Plasma triacylglycerol increased from 1.1 ± 0.3 to 1.8 ± 0.3 mmol/l and FFA increased from 0.6 ± 0.1 to 3.1 ± 0.5 mmol/l at 2 h (each P < 0.01).

**Haemodynamic data**

Basal forearm blood flow before infusion of lipid (left arm) was 2.7 ± 0.7 ml·min⁻¹·100 ml⁻¹; this increased to 3.8 ± 0.8 ml·min⁻¹·100 ml⁻¹ after 1 h of intravenous lipid (P < 0.01) and 4.7 ± 0.7 ml·min⁻¹·100 ml⁻¹ after 2 h (P < 0.01). All doses of nitroprusside increased forearm blood flow; this increment was similar at 0, 1 and 2 h (Figure 1a). All doses of acetylcholine increased forearm blood flow at each time. There was a progressive increase in response to acetylcholine during the lipid infusion (P < 0.01; Figure 1b).

**Study II**

**Metabolic data**

Glucose, insulin, LDL and HDL did not change significantly during either study day. During infusion of the lipid emulsion, triacylglycerols and FFA were increased compared with the control day (P < 0.01). Triacylglycerols increased from 1.1 ± 0.3 to a peak of 2.2 ± 0.4 mmol/l at 4 h and FFA increased from 0.5 ± 0.1 to a peak of 3.0 ± 0.4 mmol/l at 3 h (each P < 0.01).

**Haemodynamic data**

Figure 2 summarizes the haemodynamic data. During control (heparin/saline) infusion there were no significant changes in mean arterial blood pressure, heart rate or forearm blood flow. During lipid infusion, mean arterial blood pressure increased progressively by a maximum increment of 8.5 ± 2.7 mmHg at 4 h. Heart rate did not change significantly (67.7 ± 8.4 at baseline and 68.2 ± 8.5 beats·min⁻¹ at 3 h). Forearm blood flow increased from 1.73 ± 0.23 at baseline to a peak of 2.9 ± 0.3 ml·min⁻¹·100 ml⁻¹ at 3 h (P < 0.01 compared with both baseline and control day; Figure 2B). Calculated forearm vascular resistance decreased from 49.1 ± 5.4 to a nadir of 31.3 ± 3.9 arbitrary units at 3 h (P < 0.01 compared with both baseline and control day; Figure 2C).

**DISCUSSION**

Most studies exploring the effects of FFA on blood pressure homoeostasis and endothelial function have limited infusions to 1–2 h, have not carried out measurement of endothelial function at different time points, and have used varying concentrations of lipid emulsion (some producing pharmacological concentrations of FFA). We designed the present study to clarify the effects of lipid/heparin to produce pathophysiological concentra-
tions of FFA on: a) acetylcholine-mediated forearm vasodilation, and the temporal changes in any effects of exposing the vasculature to elevated FFA, and b) the effects of a more prolonged (4 h) infusion of lipid/heparin on blood pressure regulation and forearm vascular tone/blood flow. Previous studies have not explored these important points in detail nor have they used heparin/saline controls to exclude a vascular effect of heparin.

Study I

Superficially the most striking observation was the selective potentiation of acetylcholine during activation of lipolysis and increased circulating FFA caused by the lipid/heparin infusion, in agreement with some [9,10] but not all [5–8] previous studies. A more fundamental finding, however, is that lipid/heparin infusion increases basal forearm blood flow. This could explain the observed potentiation of acetylcholine, since responses to acetylcholine are directly correlated with forearm blood flow, probably as a consequence of its rapid destruction by acetylcholinesterase in blood [16–18]. This represents a potentially important caveat regarding the use of brachial artery infusion of acetylcholine as a test of endothelial function. Data from our study [18] show that the forearm blood flow responses to acetylcholine (15 μg · min⁻¹) increase by 2.1 ml · min⁻¹ · 100 ml⁻¹ for every 1 ml · min⁻¹ · 100 ml⁻¹ increase in basal forearm blood flow. The increase in basal forearm blood flow observed in the present study thus accounts for almost all of the augmented acetylcholine response observed following lipid/heparin infusion.

In the studies by Steinberg et al. [5] and Lind et al. [7] methacholine was used as a probe of endothelial function. Methacholine is more stable than acetylcholine but, unlike acetylcholine, is less susceptible to inhibition by the nitric oxide synthase inhibitor N⁰-monomethyl-L-arginine (L-NMMA) suggesting a role for vasodilating substances other than NO [19,20].

Study II

This confirmed the observation in the first study that increasing FFA increases forearm blood flow and demonstrated further that this was accompanied by an increase in mean arterial blood pressure, in agreement with others [21]. In our study the increase in mean blood pressure was probably not sufficient to account for the observed increase in forearm blood flow, as evidenced by the fall in calculated forearm vascular resistance (although local infusion of lipid/heparin into the brachial artery is needed to clarify this point). The present data are in agreement with studies in minipigs where raising circulating FFA with lipid/heparin increases arterial blood pressure and muscle blood flow [22]. The mechanism underlying the effect of lipid/heparin on blood pressure is unknown. It is possible that vasoconstriction occurs in vascular territories other than the forearm, leading to increased systemic vascular resistance [21]. It is also possible that FFA increase cardiac output through a positive inotropic effect. These effects could be important in diseases associated with increased FFA and in care of critically ill patients especially during parenteral feeding with lipid emulsion when anticoagulated with heparin [23,24].
**Study limitations**

The design of the present study does not address the mechanisms of the observed effect. However, there are a number of potential mechanisms. In addition to NO or prostaglandin mediated increments in blood flow, fatty acids may have important interactions with the renin–angiotensin [25] and reactive oxygen species generating systems [26] and to modulate the activity of the adrenergic system [27]. Dissecting out the exact mechanisms of the effect of fatty acids on blood pressure homeostasis now requires a detailed and carefully designed series of studies including measurements of cardiac output and blood flow in different vascular territories. The present dataset provides a starting point for such studies.

In conclusion, acute elevation of FFA by activating lipolysis with lipid/heparin increases arterial blood pressure and reduces vascular resistance in the forearm. These haemodynamic changes can underlie selective potentiation of acetylcholine administered by the brachial artery during elevation of plasma FFA, and could be clinically relevant.

**ACKNOWLEDGMENT**

The authors would like to thank the British Heart Foundation.

**REFERENCES**

12 Zambon, A., Hashimoto, S. I. and Brunzell, J. D. (1993) Analysis of techniques to obtain plasma for measurement of levels of free fatty acids. J. Lipid Res. 34, 1021–1028


Received 22 August 2001 | 31 October 2001; accepted 18 January 2002