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Acute elevation of plasma free fatty acids increases pulse wave velocity and induces peripheral vasodilation in humans *in vivo*

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Abstract

Plasma free fatty acid (FFA) concentrations are elevated in patients with obesity. We aimed to provide an integral haemodynamic profile of elevated plasma FFA by the simultaneous assessment of blood pressure, pulse wave velocity, forearm blood flow (FBF), and sympathetic nervous system activity during acute elevation of FFA. Secondly, we hypothesized that FFA-induced vasodilation is mediated by adenosine receptor stimulation. In a randomized crossover trial in healthy subjects, Intralipid was infused for 2 hours to elevate plasma FFA. Glycerol was administered as Control infusion. We assessed blood pressure, pulse wave velocity, FBF (venous occlusion plethysmography), and sympathetic nervous system activity by measurements of noradrenaline and adrenaline. During the last 15 minutes of Intralipid/Control infusion, the adenosine receptor antagonist caffeine (90 µg/min/dl) was administered into the brachial artery of the nondominant arm. Compared with Control infusion, Intralipid increased pulse wave velocity, systolic blood pressure and pulse pressure, as well as FBF (FBF increased from 1.8 ± 0.2 to 2.7 ± 0.6 and from 2.3 ± 0.2 to 2.7 ± 0.6 ml/min/dl for Intralipid versus Control infusion; $P < 0.05$, $n = 9$). Although in a positive control study caffeine attenuated adenosine-induced forearm vasodilation ($P < 0.01$, $n = 6$), caffeine had no effect on Intralipid-induced vasodilation ($P = 0.5$). In conclusion, elevation of plasma FFA levels increases pulse wave velocity, systolic blood pressure, and pulse pressure. Also, FBF is increased, either by baroreflex-mediated inhibition of the sympathetic nervous system, or by a direct vasodilating effect of FFA. As the adenosine receptor antagonist caffeine could not antagonize the vasodilator response, it is not mediated by adenosine receptor stimulation.

Introduction

The metabolic syndrome is characterized by (central) obesity, insulin resistance, glucose intolerance, dyslipidaemia, and hypertension. Less well-known but consistently reported features of the metabolic syndrome include a hyperdynamic circulation and sympathetic activation [1-4]. In patients with obesity and the metabolic syndrome, the plasma concentration of free fatty acids (FFA) is increased and evidence is accumulating that FFA play a central role in the clustering of the various characteristics of the metabolic syndrome [5;6]. Therefore, in the last decade, several studies explored the effect of experimental elevations of plasma FFA on haemodynamic parameters. Systemic administration of a triglyceride emulsion and heparin (to activate the lipoprotein lipase) is a well-validated and generally accepted model to study the effects of an acute elevation of plasma FFA [7-9]. Most studies agree that acute experimental elevation of FFA increases skeletal muscle blood flow [7;10-14]. Most [7;12;15-17], but not all [10;14], studies have shown an increase in mean arterial blood pressure. Also the reported effects of FFA on the activity of the sympathetic nervous system are controversial: some studies have demonstrated in rats and humans that acute elevation of FFA can increase sympathetic activity [17;18], but other studies have reported the opposite [19;20].

Recently, we presented a novel hypothesis that explains the occurrence of a hyperdynamic circulation and sympathetic activation in individuals with the metabolic syndrome [6]. We postulated that in these patients, elevated cytosolic concentrations of co-enzyme A derivatives of long-chain fatty acids (LCFA-CoA), the intracellular equivalent of FFA, inhibit the mitochondrial adenine nucleotide translocator. Consequently, intra- and extracellular concentrations of the endogenous nucleoside adenosine increase. Adenosine receptor stimulation induces various effects, including vasodilation in most vascular beds, renal sodium retention and activation of the sympathetic nervous system [21;22]. As such, elevated systemic concentrations of adenosine could induce the phenotype of a hyperdynamic circulation and sympathetic activation.

The aim of the present study is two-fold: to provide an integral haemodynamic profile of elevated plasma FFA and, more specifically, to study whether adenosine receptor stimulation is involved in the vasodilator response to FFA. Therefore, we performed a randomized controlled cross-over study in healthy volunteers in whom systemic administration of a

triglyceride emulsion (Intralipid) was used to elevate plasma FFA, and we simultaneously measured forearm blood flow (FBF), intra-arterial blood pressure, pulse wave velocity, and estimated the activity of the sympathetic nervous system by plasma noradrenaline measurements. In addition, the adenosine receptor antagonist caffeine was administered into the brachial artery of the non-dominant arm to study whether the FFA-induced increase in FBF is due to direct adenosine receptor stimulation in the forearm vascular bed.

Methods

Subjects

The study conformed with the principles outlined in the Declaration of Helsinki and was approved by the Institutional Review Board of the Radboud University Nijmegen Medical Centre. Fifteen young healthy volunteers signed written informed consent before participation. All participants underwent a physical examination and electrocardiography to exclude hypertension and cardiovascular or pulmonary disease. As caffeine is an effective adenosine receptor antagonist [23], all volunteers were asked to abstain from caffeine-containing beverages for at least 24 hours before the study.

Instrumentation

All experiments were performed in the morning after an overnight fast in a temperature-controlled room (23 °C) with the subjects in supine position. After local anaesthesia (xylocaine 2%), the brachial artery of the non-dominant arm was cannulated with a 20-gauge catheter (Angiocath, Becton Dickinson, Sandy, UT, USA) for intra-arterial administration of caffeine (automatic syringe infusion pump, type STC-521, Terumo Corporation, Tokyo, Japan) and blood pressure recording (Hewlett Packard GmbH, Böblingen, Germany). Forearm blood flow (FBF) was recorded simultaneously on both arms by venous occlusion plethysmography using mercury-in-silastic strain gauges (Hokanson EC4, Hokanson, Inc., Washington, USA), as previously described [24]. Before each recording a wrist cuff was inflated to 200 mmHg to exclude the hand circulation.

In the main study two catheters were inserted into large antecubital veins of both arms. Intralipid or glycerol was administered intravenously into the dominant arm (vide infra). Venous blood was drawn from the non-dominant arm for the determination of FFA, glycerol, triglycerides, and glucose. Arterial and venous adrenaline and noradrenaline levels were assessed in blood simultaneously drawn from the brachial artery and antecubital vein from the non-dominant arm. Plasma insulin concentration was determined in arterial blood.

Validation study

Six healthy volunteers participated in the validation study. Thirty minutes after cannulation of the brachial artery of the non-dominant arm, FBF was measured for 5 minutes during saline infusion. Then, adenosine was administered into the brachial artery (5 µg/min per dl of

forearm tissue) for 10 minutes and FBF was measured. After 20 minutes wash-out, adenosine infusion was repeated, 5 minutes later followed by co-infusion of caffeine (90 µg/min/dl).

Main study

For this study 9 subjects were studied twice according to a randomized crossover design. All subjects were asked to avoid excessive exercise for 24 hours before the tests and to have a similar dinner the evening before both experiments. The second experiment was conducted one month after the first. In one experiment (Intralipid experiment), subjects received an intravenous dose of a triglyceride emulsion (Intralipid[®] 20%, 90 ml/h) with co-administration of heparin (bolus 500 IU, followed by continuous infusion of 0.2 IU/kg/min intravenously) to activate lipoprotein lipase [7]. In the other experiment (Control experiment), glycerol (50% solution; 3 gram/h) and heparin were administered to control for the elevated glycerol and heparin concentration during the Intralipid experiment. This design has previously been described by Krebs *et al* [25].

In both experiments, FBF was measured for 5 minutes during saline infusion, thirty minutes after cannulation. Thereafter, Intralipid or Control infusion was started. In both experiments, FBF was recorded for 5 minutes and blood was drawn for the determination of FFA, triglycerides and glycerol 60 and 120 minutes after initiation of infusion. Concentrations of insulin, adrenaline, and noradrenaline were determined on t=0 and t=120 minutes. Finally, caffeine was administered for 15 minutes into the brachial artery (90 µg/min/dl) during continuous Intralipid or Control infusion and with uninterrupted measurement of FBF.

During the 10 minutes immediately before the start of administration of Intralipid/Control and during the last 10 minutes of infusion, heart rate was recorded by surface electrocardiography, and blood pressure was recorded in the brachial artery. For each heartbeat, we calculated the time difference between the electrocardiographic R wave and arrival of the pressure wave at the brachial artery and averaged this value over these 10 minute periods (MIDAC test-organiser, Radboud University Nijmegen Medical Centre). The pressure wave signal was first filtered with a lowpass filter (first order, 10 Hz), after which the local maxima (systole) and minima (diastole) were determined. Subsequently, for each pressure wave, the best line was fit with linear regression through the rising edge between 10% and 90% of the maximum-minimum amplitude. The intersection between this line and a horizontal line (placed at the level of the diastolic value) was taken as the arrival of the bloodpressure wave. As the travel

distance is the same for each subject during the two experiments, this time difference can be considered as an measure for pulse wave velocity.

Analytical procedures

Blood samples for the determination of glycerol, FFA and triglycerides were collected in pre-cooled EDTA-containing vacutainers and centrifuged at 1000 *g* for 10 min at 4°C. Aliquots of plasma were immediately stored at –80°C. FFA (NEFA-C; Wako Chemicals, Neuss, Germany), free glycerol (148270; Roche Diagnostics, Indianapolis, IN), and triglyceride (GPO-Trinder 337B; Sigma Diagnostics, St Louis, MO) concentrations were analyzed with a COBAS semiautomatic analyzer (Roche). Due to technical problems, baseline plasma glucose levels were only determined in our laboratory in the first five experiments.

For the determination of plasma insulin, adrenaline, and noradrenaline, blood was collected in pre-cooled lithium-heparin-containing vacutainers. Plasma concentrations of noradrenaline and adrenaline were determined by HPLC with fluorimetric detection [26]. Insulin was measured with an insulin-specific double antibody radioimmunoassay (interassay coefficient of variation, 6.2%). Plasma caffeine concentrations were determined by reversed-phase HPLC with UV-detection set at 273 nm.

Forearm spillover of noradrenaline was estimated as previously described by Rongen *et al* [27]. To correct for changes in FBF, we also calculated appearance rate of noradrenaline [27;28]. For these calculations, forearm fractional extraction of adrenaline was used as a substitute for noradrenaline extraction.

Statistical analysis

All data are expressed as mean \pm SE. Baseline parameters of subjects in the validation study and main study were compared with an unpaired Student's *t*-test and baseline values in both experiments of the main study were compared with a paired Student's *t*-test. Plasma concentrations of free fatty acids, glycerol, triglycerides and insulin as well as haemodynamic parameters in both groups were analyzed by repeated measures ANOVA. Forearm vascular resistance was calculated by the ratio of mean arterial pressure and FBF. SPSS for Windows (release 12.0.1) was used for statistical analyses.

Results

Baseline characteristics

There were no significant differences between subjects participating in the validation study and the main study. Average age was 21.2 ± 0.6 years. Baseline plasma caffeine concentration was 0.29 ± 0.17 mg/l in the validation study and 0.21 ± 0.10 versus 0.15 ± 0.07 mg/l in the Intralipid and Control study, respectively.

Validation study

Infusion of adenosine into the brachial artery of the non-dominant arm for 5 minutes increased FBF from 1.9 ± 0.4 to 4.0 ± 0.7 ml/min per dl of forearm tissue during the first period of adenosine administration and to 4.1 ± 0.8 ml/min/dl during the second period (figure 1a). In the first period, adenosine infusion was continued resulting in a further increase in FBF up to 6.3 ± 1.0 ml/min/dl. In the second period, caffeine was co-administered after 5 minutes, resulting in a decrease of FBF to 2.9 ± 0.5 ml/min/dl. There was a significant difference between the change in FBF during the first 5 minutes of caffeine administration and the change in FBF observed during continuous infusion of adenosine alone (figure 1b; $P < 0.01$). There were no significant differences between both infusion periods regarding blood pressure, heart rate and FBF in the control arm (data not shown). These results show that caffeine in the dose administered is effective in reducing adenosine-induced vasodilation.

Main study

Haemodynamic values and plasma concentrations of FFA, glycerol, triglycerides, glucose and insulin at baseline, and after 1 and 2 hours of Intralipid/Control infusion are summarized in table 1. Plasma concentrations of FFA, triglycerides and glycerol increased significantly more during administration of Intralipid than during Control infusion. During Intralipid infusion a small yet significant increase in plasma insulin concentration was observed (table 1).

Both Intralipid and Control infusion resulted in a significant increase in systolic blood pressure, pulse pressure and heart rate ($P < 0.01$ for all three parameters), whereas diastolic blood pressure and mean arterial pressure were not affected. However, increases in systolic blood pressure and pulse pressure were significantly more pronounced during Intralipid infusion (table 1).

Baseline FBF was significantly higher before Control infusion than before Intralipid infusion. Therefore, we calculated the change in FBF from baseline to express the effects of each intervention. Administration of Intralipid resulted in a significant increase in FBF compared with Control infusion (Figure 2; $P < 0.05$). Also, FVR (MAP/FBF) was significantly decreased during Intralipid infusion compared with Control infusion (from 52.3 ± 5.1 to 40.3 ± 5.3 and from 39.2 ± 3.6 to 43.2 ± 7.1 for Intralipid and Control, respectively, $P < 0.05$).

The time difference between the electrocardiographic R wave and arrival of the pressure wave at the brachial artery decreased during Intralipid administration compared with Control infusion (table 1), indicating that Intralipid administration significantly increased pulse wave velocity. We used the baseline measurements of the time difference in both groups (before any intervention) to calculate the coefficient of variation for this technique, which was 6.5% (average of standard deviation divided by mean for each subject). For biological variables, a coefficient of variation of $<10\%$ is considered good, and $<20\%$ is acceptable [29].

Administration of Intralipid had neither an effect on arterial noradrenaline concentration nor on calculated forearm noradrenaline spillover compared with Control infusion. If anything, forearm appearance rate of noradrenaline tended to increase more during Control infusion (table 2).

During continuous systemic administration of Intralipid, infusion of caffeine into the brachial artery of the non-dominant arm did not have any effect on FBF (figure 3; $P = 0.5$ compared with the control arm). Also during continuous Control infusion, caffeine did not affect FBF (data not shown).

Discussion

The main finding of this study is that acute elevation of FFA induces a haemodynamic profile consisting of increased pulse wave velocity, elevated systolic blood pressure and pulse pressure and peripheral vasodilation. The observed FFA-induced forearm vasodilation is not mediated by adenosine receptor stimulation.

Previous studies have shown that a hyperdynamic circulation is associated with several characteristics of the metabolic syndrome, although these findings have not received much attention. In adults a hyperdynamic circulation is associated with increased body mass index, triglyceride levels, glucose and insulin levels [1]. Moreover, insulin resistance and obesity are associated with increased arterial stiffness [30;31], which is an important cardiovascular risk marker [32].

We hypothesized that this hyperdynamic circulation is caused by increased concentrations of plasma FFA [6]. In obese subjects the release of FFA into the circulation is increased compared with lean subjects [33]. Indeed, we demonstrated that acute experimental elevation of plasma FFA increased systolic blood pressure and pulse pressure, but we did not observe an increased activity of the sympathetic nervous system. More specifically, we hypothesized that FFA would induce a hyperdynamic circulation and sympathetic activation by increased adenosine receptor stimulation [6]. In animal studies, LCFA-CoA have been shown to result in a concentration-dependent inhibition of the mitochondrial adenine nucleotide translocator in a physiological concentration range [34]. The adenine nucleotide translocator transports ADP from the cytosol into the mitochondrial matrix in exchange for ATP. As such, inhibition of this translocator results in increased cytosolic ADP concentrations. Consequently, according to the cytosolic adenylate kinase equilibrium, ATP and AMP concentrations increase and AMP is converted into adenosine. Indeed, it was shown *in vitro* that palmitoyl-CoA inhibits the adenine nucleotide translocator and increases extramitochondrial AMP concentrations [35]. An increased intracellular adenosine concentration diminishes the transmembranous adenosine concentration gradient, thus limiting uptake of extracellular adenosine [36]. By stimulation of specific membrane-bound adenosine receptors, adenosine induces various cardiovascular and neurohumoral effects [21], such as vasodilation, and activation of the sympathetic nervous system. In addition, some studies suggest that increased systemic adenosine concentrations result in renal sodium and water retention [22]. Together,

these findings suggest a role for adenosine in causing the haemodynamic changes observed in the metabolic syndrome and in response to administration of FFA.

It has been demonstrated previously that acute elevation of FFA is associated with increased forearm and leg blood flow [7;10-14]. In the present study we confirmed these findings. Subsequently, we administered the adenosine receptor antagonist caffeine into the brachial artery of the non-dominant arm in order to explore the role of local adenosine receptor stimulation in this FFA-induced vasodilation. Administration of caffeine did not affect FBF in this setting, whereas in the same dose it effectively blocked adenosine-induced vasodilation. As such, our findings do not support a role for adenosine in the vasodilator action of FFA. Unfortunately, we did not measure endogenous plasma adenosine concentrations during administration of Intralipid. It is complicated to measure adenosine, because of the extremely short half life of less than 1 second [37]. It is possible to estimate adenosine concentration by microdialysis [38], but this technique lacks sensitivity to detect small differences in adenosine concentration.

In the present study, experimental elevation of FFA was not associated with activation of the sympathetic nervous system. If anything, a reduction of forearm appearance rate of noradrenaline was observed. Previous studies on this topic in healthy subjects yielded inconsistent results [17;20]. Also in rat models, administration of lipid emulsions has been demonstrated to either increase [18] or decrease [19] sympathetic activity. Because of these inconsistent results, it remains to be determined whether FFA are responsible for the reported association between the metabolic syndrome and sympathetic activation.

Interestingly, we also demonstrated that administration of Intralipid decreased the time difference between the electrocardiographic R wave and arrival of the pressure wave at the brachial artery. As the travel distance is the same for each subject during both experiments, this observation implies that elevation of FFA increases pulse wave velocity. This is in accordance with the results of two previous studies in which compliance after administration of a triglyceride emulsion was estimated by pulse wave analysis [16;39].

A plausible explanation for the haemodynamic effects of FFA in our study is that FFA increased pulse wave velocity by decreasing arterial compliance, which increased systolic

blood pressure and pulse pressure by impairing windkessel function and by summation of the propagated and reflected pressure waves. Subsequently, baroreflex-mediated inhibition of the sympathetic nervous system resulted in peripheral vasodilation. A concomitant reduction in heart rate would have been expected in this situation, but it has been demonstrated in rats [40] and humans [39] that acute experimental elevation of plasma FFA reduces baroreflex sensitivity, which is considered mainly to reflect vagal activity. An alternative explanation would be that FFA also have a direct vasodilating effect on resistance arteries, not mediated by adenosine receptor stimulation, in addition to the reduction in arterial compliance. Finally, a direct effect of Intralipid administration on cardiac stroke volume could have contributed to the observed increase in pulse pressure.

A number of potential limitations of our study should be mentioned. First, the study was not blinded. Because of the different colour of the Intralipid emulsion and glycerol solution blinding of this study would have been extremely complicated. However, the test sequence was randomized, and the haemodynamic and laboratory analyses were performed blinded. Second, plasma insulin levels were slightly but significantly increased by administration of Intralipid compared with Control. This is in accordance with the well-known effect of FFA on insulin-mediated glucose uptake [25]. It is unlikely that this increased insulin concentration contributed to the increase in FBF because in previous studies with comparable study protocols, insulin either slightly increased [10] or did not increase at all [14], whereas blood flow increased in all studies. Moreover, insulin-mediated vasodilation was observed in previous studies only after much higher elevations of plasma insulin of approximately ten-times baseline levels [41]. Third, baseline FBF was significantly higher before Control infusion than before Intralipid infusion. However, it is unlikely that this affected the vasomotor response to Intralipid/Control infusion, as there was no correlation between baseline FBF and the change in FBF in response to Intralipid or Control infusion (data not shown). Fourth, in both groups, parameters of sympathetic activity tended to increase in time (table 2). Also, systolic blood pressure and heart rate significantly increased in time in both groups. This could be stress-related, caused by discomfort of the experimental procedure. As the study had a randomized cross-over design, it is unlikely that this would have influenced our results. Finally, it has to be realized that we studied acute and not chronic elevation of plasma FFA and that the plasma FFA concentration after Intralipid infusion was higher than the FFA concentration in patients with the metabolic syndrome or diabetes mellitus [42].

Thus, future studies are warranted to confirm that the haemodynamic effects as observed in the present study also apply to chronic and less extreme elevations in plasma FFA concentration.

In conclusion, acute elevation of plasma FFA levels in healthy young subjects increased pulse wave velocity, systolic blood pressure, and pulse pressure, decreased forearm vascular resistance, but did not significantly increase the activity of the sympathetic nervous system. The reduced vascular resistance could be due to baroreflex-mediated inhibition of sympathetic nerve activity. Alternatively, FFA could have direct vasodilator effects, which, however, are not mediated by local adenosine receptor stimulation.

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Figure Legends

Figure 1

FBF in the experimental arm in the pilot-study during administration of adenosine (filled circles) and adenosine/cafeine (open circles) in the brachial artery (a, n=6, mean \pm SE). Figure 1b depicts the change in FBF after the addition of cafeine (open circles) or continued administration of adenosine only (filled circles). $P<0.01$ for difference between both interventions, ANOVA for repeated measures.

Figure 2

FBF in the experimental arm, expressed as change from baseline, during Intralipid (filled circles) and Control infusion (open circles). $P<0.05$ for difference between both interventions, ANOVA for repeated measures (n=9, mean \pm SE).

Figure 3

FBF in the experimental (filled circles) and control (open circles) arm during continuous administration of cafeine into the brachial artery for 15 minutes during continuous systemic Intralipid administration. $P=0.5$ for comparison between both arms, ANOVA for repeated measures (n=9, mean \pm SE).

Table 1: Haemodynamic and laboratory values in the main study (mean \pm SE; I = Intralipid; C = Control)

Parameter		baseline	1 hour	2 hours
FBF (ml/min/dl)	I	1.8 \pm 0.2	2.1 \pm 0.3	2.7 \pm 0.6
	C	2.3 \pm 0.2	2.2 \pm 0.3	2.7 \pm 0.6
		* †		
MAP (mmHg)	I	80 \pm 2	81 \pm 2	84 \pm 2
	C	82 \pm 3	83 \pm 3	84 \pm 3
SBP (mmHg)	I	115 \pm 3	119 \pm 3	122 \pm 3
	C	116 \pm 3	118 \pm 3	120 \pm 3
		#		
DBP (mmHg)	I	62 \pm 1	62 \pm 2	64 \pm 2
	C	63 \pm 3	63 \pm 3	65 \pm 3
Pulse Pressure (mmHg)	I	53 \pm 2	58 \pm 2	59 \pm 2
	C	53 \pm 2	55 \pm 2	55 \pm 2
		†		
Heart rate (bpm)	I	59 \pm 2	61 \pm 2	65 \pm 2
	C	59 \pm 2	60 \pm 2	63 \pm 2
Pulse wave time to brachial artery (s)	I	174 \pm 6		156 \pm 6
	C	188 \pm 18		184 \pm 18
		†		
FFA (μ mol/l)	I	334 \pm 36	965 \pm 66	1269 \pm 78
	C	338 \pm 38	393 \pm 47	432 \pm 58
		†		
Triglycerides (μ mol/l)	I	746 \pm 72	2824 \pm 256	3947 \pm 558
	C	788 \pm 148	842 \pm 130	912 \pm 137
		†		
Glycerol (μ mol/l)	I	68 \pm 8	333 \pm 33	470 \pm 47
	C	63 \pm 7	301 \pm 23	307 \pm 43
		†		
Glucose (mmol/l)	I	5.0 \pm 0.0		4.5 \pm 0.2
	C	4.7 \pm 0.1		4.5 \pm 0.0
Insulin (pmol/l)	I	42.0 \pm 5.6		49.2 \pm 4.3
	C	40.8 \pm 4.0		37.8 \pm 3.6
		†		

*P<0.05 for difference between I and C at baseline. #P=0.05 and †P<0.05 for difference between I and C (repeated measures ANOVA).

Table 2: Plasma noradrenaline kinetics (mean \pm SE)

Parameter	Intralipid T = 0	Intralipid T = 120	Control T = 0	Control T = 120
Arterial noradrenaline (nmol/l)	0.63 \pm 0.06	0.71 \pm 0.11	0.57 \pm 0.07	0.70 \pm 0.10
Arterial adrenaline (nmol/l)	0.21 \pm 0.03	0.17 \pm 0.02	0.17 \pm 0.02	0.25 \pm 0.04
Spillover (pmol/min/dl forearm tissue)	0.66 \pm 0.18	0.85 \pm 0.21	0.84 \pm 0.16	1.06 \pm 0.12
Appearance rate (pmol/min/dl forearm tissue)	2.10 \pm 0.59	2.82 \pm 0.82	2.30 \pm 0.70	3.30 \pm 0.59*

* P = 0.07 for comparison Intralipid versus Control

Figure 1

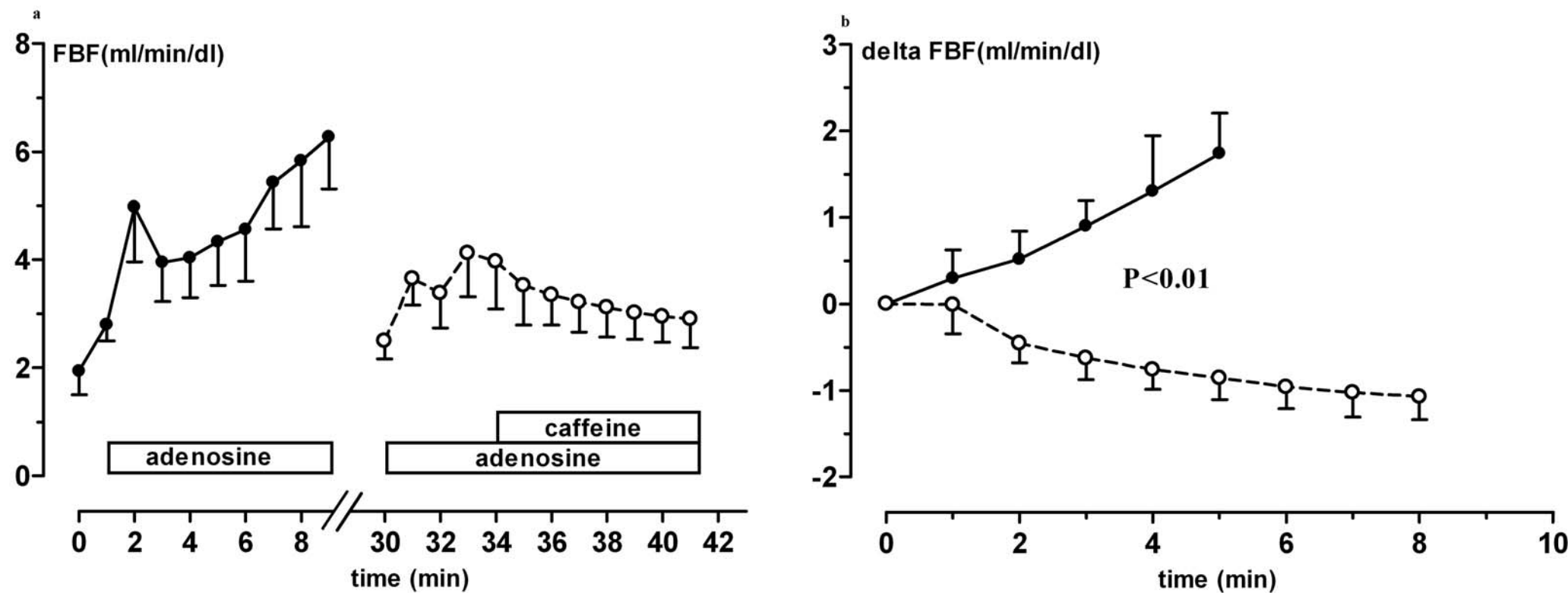


Figure 2

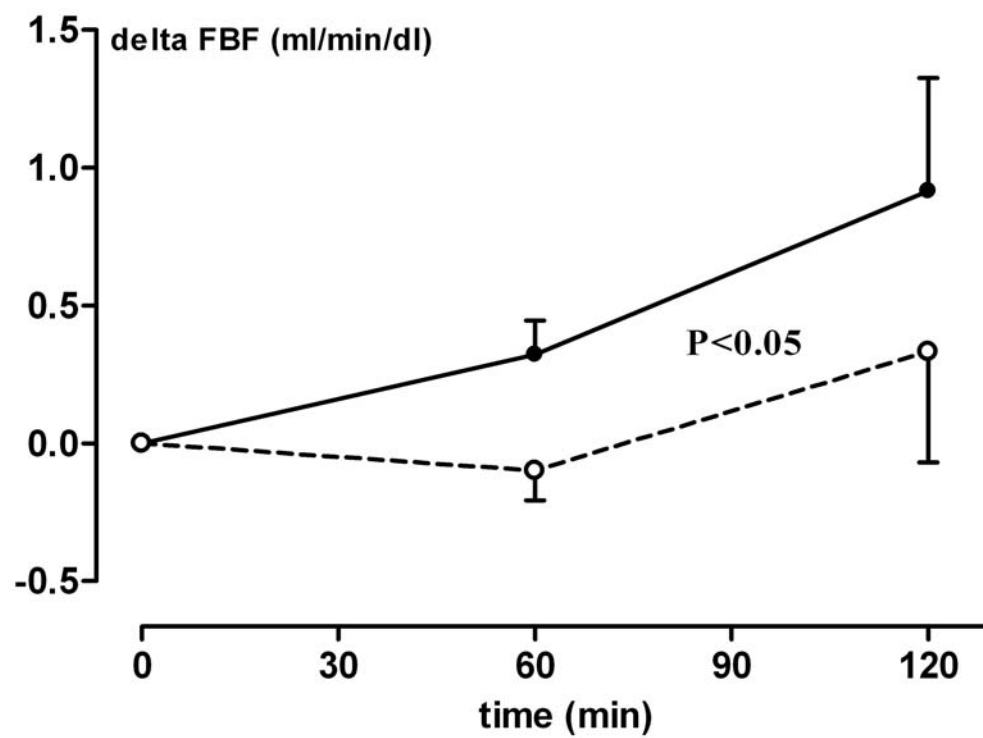


Figure 3

