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Citation for published version (APA):

Schiffelers, S. L. H., Brouwer, E. M. C., Saris, W. H. M., & van Baak, M. A. (1998). Inhibition of lipolysis reduces beta1-adrenoceptor-mediated thermogenesis in man. *Metabolism-Clinical and Experimental*, 47(12), 1262-1267. [https://doi.org/10.1016/S0026-0495\(98\)90070-1](https://doi.org/10.1016/S0026-0495(98)90070-1)

Document status and date:

Published: 01/01/1998

DOI:

[10.1016/S0026-0495\(98\)90070-1](https://doi.org/10.1016/S0026-0495(98)90070-1)

Document Version:

Publisher's PDF, also known as Version of record

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Inhibition of Lipolysis Reduces β_1 -Adrenoceptor-Mediated Thermogenesis in Man

S.L.H. Schiffelers, E.M.C. Brouwer, W.H.M. Saris, and M.A. van Baak

The purpose of the study was to investigate whether the increase in energy expenditure and lipid oxidation during β_1 -adrenergic stimulation is caused by the concomitant increase in lipolysis. Twelve healthy male subjects participated in three trials: no-LIP/-, inhibition of lipolysis by pretreatment with acipimox followed by saline infusion; -/BETA, no pretreatment, with dobutamine infusion to stimulate β_1 -adrenoceptors; and no-LIP/BETA, pretreatment with acipimox followed by dobutamine infusion. Inhibition of lipolysis did not affect baseline energy expenditure, but decreased lipid oxidation and increased carbohydrate oxidation. Energy expenditure and lipid oxidation increased significantly during β_1 -adrenergic stimulation, but this increase was significantly smaller when lipolysis was inhibited ([baseline v infusion period] energy expenditure: -/BETA, 5.15 ± 0.16 v 6.11 ± 0.26 kJ/min, $P < .001$; no-LIP/BETA, 5.28 ± 0.17 v 5.71 ± 0.19 kJ/min, $P < .01$; lipid oxidation: -/BETA, 0.059 ± 0.004 v 0.073 ± 0.006 g/min, $P < .01$; no-LIP/BETA, 0.034 ± 0.005 v 0.039 ± 0.006 g/min, $P < .05$). Baseline plasma glycerol and nonesterified fatty acid (NEFA) concentrations decreased after inhibition of lipolysis. Glycerol and NEFA increased significantly during β_1 -adrenergic stimulation alone (glycerol, 65.0 ± 5.3 v 117.0 ± 10.9 $\mu\text{mol/L}$; NEFA, 362 ± 24 v 954 ± 89 $\mu\text{mol/L}$; both $P < .001$). Concomitant administration of acipimox prevented a substantial part of the increase in lipolysis during β_1 -adrenergic stimulation, but the increase in plasma glycerol and NEFA remained significant (glycerol, 40.4 ± 2.2 v 44.8 ± 2.2 $\mu\text{mol/L}$; NEFA, 118 ± 18 v 160 ± 19 $\mu\text{mol/L}$; both $P < .05$). In conclusion, a reduced availability of plasma NEFA was associated with a reduced increase in energy expenditure and lipid oxidation during β_1 -adrenergic stimulation in man.

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THE SYMPATHETIC NERVOUS system plays a role in energy metabolism. Previous studies in humans have shown that mainly β_1 - and β_2 -adrenoceptors are involved in sympathetically mediated thermogenesis, whereas α_1 - and α_2 -adrenoceptors do not have an important role.^{1,2} The role of the β_3 -adrenoceptor in human thermogenesis is still debated,^{3,4} and this will be the case until a specific agonist or antagonist for the human β_3 -adrenoceptor is available.

It is still uncertain which processes are responsible for the sympathetically mediated thermogenesis and in which tissues these processes are localized. β_1 -adrenoceptors are found in adipose tissue, heart, and brain, while β_2 -adrenoceptors are present in skeletal muscle, blood vessels, and liver. Infusion of the endogenous catecholamines epinephrine and norepinephrine leads to an increase in adipose tissue lipolysis^{5,6} and whole-body^{7,8} and skeletal muscle⁵ lipid oxidation. Infusion of the nonselective β -agonist isoprenaline also leads to an increase in lipolysis and lipid oxidation.⁹

Recent studies showed an increase in lipolysis, lipid oxidation, and energy expenditure during β_1 -adrenergic stimulation with dobutamine.^{10,11} The increase in lipolysis can be explained by the presence of β_1 -adrenoceptors in adipose tissue. The increase in lipid oxidation and thermogenesis is assumed to be localized predominantly in skeletal muscle,^{5,9} but this tissue contains mainly β_2 -adrenoceptors and presumably no β_1 -adrenoceptors.¹² β_1 -Adrenergic stimulation therefore is not

likely to increase energy expenditure and lipid oxidation by direct stimulation of skeletal muscle. We therefore hypothesize that the elevated levels of nonesterified fatty acid (NEFA) in blood (caused by increased lipolysis due to β_1 -adrenoceptor stimulation) induce the increase in energy expenditure and lipid oxidation during β_1 -adrenergic stimulation. To investigate this possibility, we inhibited lipolysis with acipimox (a long-acting nicotinic acid derivate) and stimulated the β_1 -adrenoceptors with dobutamine to determine whether a reduced availability of plasma NEFA would result in a reduced increase in energy expenditure and lipid oxidation.

SUBJECTS AND METHODS

Subjects

Twelve male subjects aged 25.0 ± 1.6 years (mean \pm SEM; range, 19 to 41) participated in the study. Their body weight was 73.8 ± 3.0 kg (range, 57.2 to 93.1), body mass index 22.8 ± 0.8 kg/m² (range, 18.5 to 27.8), and fat percentage $12.3\% \pm 1.6\%$ (range, 5.2% to 22.4%). The subjects were healthy and taking no medication at the time of study. The study was reviewed and approved by the Ethics Committee of Maastricht University, and all subjects provided informed consent before participating.

Experimental Design

The subjects participated in three trials performed in random order. In trial no-LIP/-, lipolysis was inhibited by pretreatment with acipimox (Nedios; Byk Nederland, Zwanzburg, The Netherlands), after which saline was given as a placebo infusion. In trial -/BETA, no pretreatment was given; only dobutamine (Dobax; Byk Nederland) was infused to stimulate β_1 -adrenoceptors. In trial no-LIP/BETA, pretreatment with acipimox was followed by infusion with dobutamine. No placebo capsule was given for acipimox, since blinding could not be obtained. The flushing observed after acipimox would clearly distinguish acipimox from placebo. The study design was single-blind for dobutamine. Subjects were fasted overnight and arrived at the laboratory by car or bus to minimize the amount of physical activity before the tests. Experiments started at 8:00 or 9:30 AM, with each subject always starting at the same time. At least 3 days separated consecutive tests. Room temperature was kept at 21° to 23°C.

The capsule of acipimox (250 mg) was administered 90 minutes

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Submitted November 19, 1997; accepted June 24, 1998.

Supported by a grant from The Netherlands Organization for Scientific Research (903-39-138).

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0026-0495/98/4712-0006\$03.00/0

before the start of the experiment (Fig 1). On arrival at the laboratory, a cannula was inserted into a forearm vein, after which measurements of the energy expenditure, respiratory exchange ratio (RER), heart rate, and blood pressure were started with the subject in a semisupine position. After a 30-minute baseline period, a continuous infusion of dobutamine ($5 \mu\text{g}/\text{kg}$ body weight \cdot min) or saline was administered for 30 minutes. At the end of each 30-minute period, a venous blood sample was taken. The infusion was stopped prematurely if the heart rate increased by more than 30 beats per minute (bpm) and/or mean blood pressure increased by more than 30 mm Hg. This occurred once during the study.

Clinical Methods

Body density was determined by hydrostatic weighing with simultaneous lung volume measurement (Volugraph 2000; Mijndhardt, The Netherlands). Body composition was calculated according to the Siri formula.¹³ The whole-body energy expenditure and RER were measured by a home-made open-circuit ventilated-hood system. The volume of air drawn through the hood was measured by a dry-gas meter (Schlumberger, Dordrecht, The Netherlands), and the composition of inflowing and outflowing air was analyzed by a paramagnetic O_2 analyzer (Servomex, Crowborough, UK) and an infrared CO_2 analyzer (Hartmann and Braun, Frankfurt, Germany). The airflow rate and O_2 and CO_2 concentration of the inflowing and outflowing air were used to compute O_2 consumption and CO_2 production on-line through an automatic acquisition system connected to a personal computer. The energy expenditure and RER were calculated according to the formula proposed by Weir.¹⁴ Energy expenditure and RER values were averaged over the last 10 minutes of each 30-minute period during which a steady state occurred. Carbohydrate and lipid oxidation rates were calculated from O_2 consumption and CO_2 production rates as described by Ferrannini,¹⁵ assuming that protein oxidation accounted for 15% of energy expenditure, and were averaged over the last 10 minutes of each 30-minute period. The heart rate was monitored continuously by conventional electrocardiography and recorded at the end of each 5-minute interval. Heart rate values were averaged over the last 10 minutes of each 30-minute period and used for further analysis. Blood pressure was measured by an automated blood pressure device (Tonoprint; Speidel & Keller, Jungingen, Germany) after 20 minutes in each 30-minute interval. The mean values of four measurements per interval were computed and used for further analysis.

Analytical Methods

Blood samples were preserved in sodium-EDTA ($20 \mu\text{L}$ 7.5%-wt/vol Na-EDTA per 1 mL blood) and immediately centrifuged for 10 minutes at 3,000 rpm at 4°C . The plasma was transferred to microtest tubes,

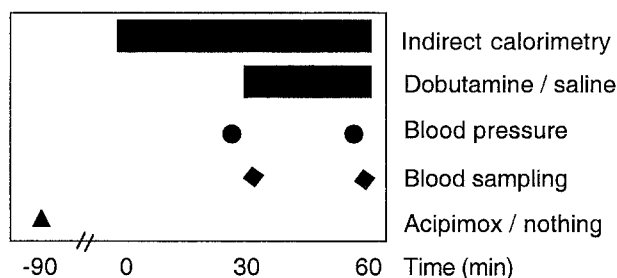


Fig 1. Experimental study design. Twelve male subjects participated in 3 randomized trials: no-LIP/-, inhibition of lipolysis by pretreatment with acipimox followed by saline infusion; -/BETA, no pretreatment, with dobutamine infusion to stimulate β_1 -adrenoceptors; no-LIP/BETA, pretreatment with acipimox followed by dobutamine infusion.

rapidly frozen in liquid nitrogen, and stored at -60°C until further analysis. The plasma NEFA concentration was measured with a NEFA C kit (99475409; Wako, Neuss, Germany), and the plasma glycerol concentration was measured with a glycerol kit (148270; Boehringer, Mannheim, Germany), both on a Cobas-Fara centrifugal analyzer (Roche Diagnostica, Basel, Switzerland). The plasma glucose concentration was measured with a glucose kit (Unimate 5, 0736724; Roche), and plasma lactate was determined by the method of Gutmann and Wahlefeld,¹⁶ both on a Cobas-Bio centrifugal analyzer. Plasma insulin was determined with a double-antibody radioimmunoassay (Insulin RIA 100; Pharmacia, Uppsala, Sweden).

Data Analysis

The data are presented as the mean \pm SEM. Comparison of data between the baseline and infusion periods within a trial was made with a Student paired *t* test. Comparison of data at baseline or during the infusion period among trials was performed with a repeated-measures ANOVA. Post hoc testing was performed with a Student paired *t* test, and *P* values for post hoc comparisons were corrected according to Bonferroni's inequalities. A *P* value less than .05 was regarded as statistically significant.

RESULTS

Energy Expenditure, RER, and Substrate Oxidation

Inhibition of lipolysis did not affect energy expenditure at baseline (Fig 2). Energy expenditure increased from 5.15 ± 0.16 to 6.11 ± 0.26 kJ/min ($P < .001$) during β_1 -adrenergic stimulation alone, and from 5.28 ± 0.17 to 5.71 ± 0.19 kJ/min ($P < .01$) during β_1 -adrenergic stimulation with concomitant inhibition of lipolysis. The increase in energy expenditure was significantly reduced when lipolysis was inhibited (-/BETA v no-LIP/BETA, 0.93 ± 0.15 v 0.43 ± 0.10 kJ/min, $P < .05$). The RER increased significantly at baseline when lipolysis was inhibited (Fig 2). The RER decreased during the infusion period in all trials, but this decrease was only significant during β_1 -adrenergic stimulation alone.

Baseline carbohydrate oxidation was significantly higher and baseline lipid oxidation was significantly lower after inhibition of lipolysis (Fig 3). Carbohydrate and lipid oxidation did not change significantly during saline infusion. Carbohydrate oxidation also did not change during β_1 -adrenergic stimulation with or without concomitant inhibition of lipolysis. Lipid oxidation increased from 0.059 ± 0.004 to 0.073 ± 0.006 g/min ($P < .01$) during β_1 -adrenergic stimulation alone. When lipolysis was inhibited, lipid oxidation increased from 0.034 ± 0.005 to 0.039 ± 0.006 g/min during β_1 -adrenergic stimulation ($P < .05$). The increase in lipid oxidation was significantly reduced when lipolysis was inhibited (-/BETA v no-LIP/BETA, 0.015 ± 0.004 v 0.006 ± 0.002 g/min, $P < .05$).

Blood Parameters

Baseline plasma concentrations of glycerol and NEFA decreased significantly after inhibition of lipolysis (Fig 4). The plasma glycerol concentration did not change during saline infusion, but plasma NEFA decreased slightly further from 113 to $93 \mu\text{mol}/\text{L}$ ($P < .001$). Plasma glycerol and NEFA increased significantly during β_1 -adrenergic stimulation alone (glycerol, 65.0 ± 5.3 v $117.0 \pm 10.9 \mu\text{mol}/\text{L}$; NEFA, 362 ± 24 v $954 \pm 89 \mu\text{mol}/\text{L}$; both $P < .001$). After inhibition of lipolysis, plasma glycerol and NEFA still increased slightly but significantly

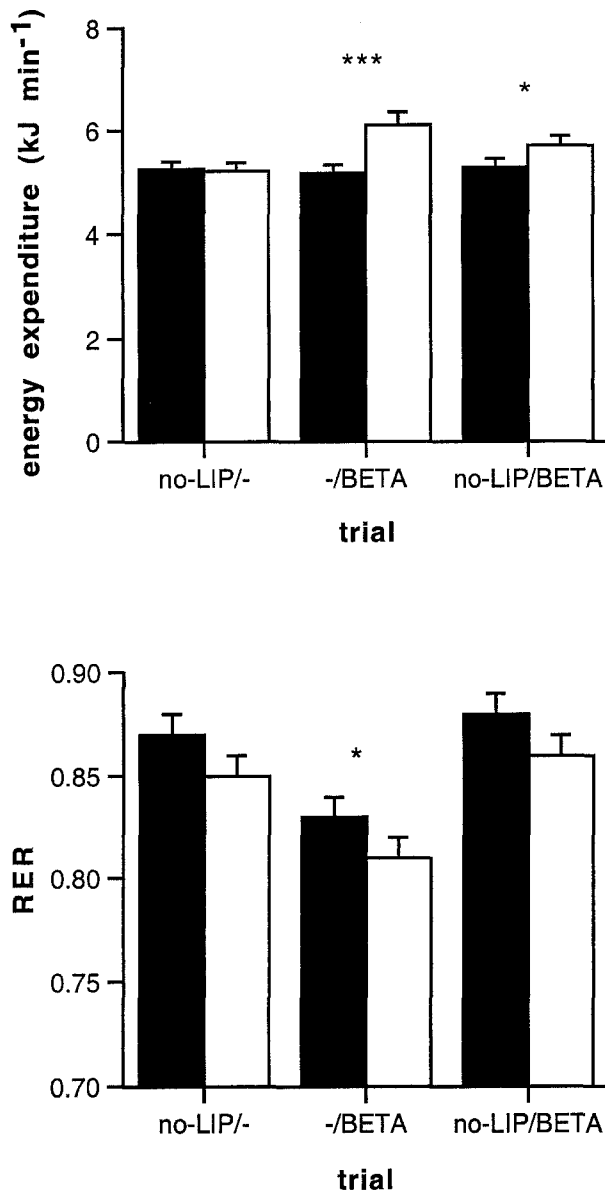


Fig 2. Energy expenditure and RER (mean \pm SEM, N = 12) during baseline period (■) with or without inhibition of lipolysis (no-LIP) by acipimox and during infusion (□) of saline or dobutamine to stimulate β_1 -adrenoceptors (BETA). Student paired *t* test corrected for Bonferroni inequalities, baseline *v* infusion period: **P* < .05, ****P* < .001.

during β_1 -adrenergic stimulation (glycerol, 40.4 ± 2.2 *v* 44.8 ± 2.2 $\mu\text{mol/L}$; NEFA, 118 ± 17 *v* 160 ± 19 $\mu\text{mol/L}$; both *P* < .05). The increases in plasma glycerol and NEFA were significantly reduced when lipolysis was inhibited (-/BETA *v* no-LIP/BETA: glycerol, 52.0 ± 10.2 *v* 4.4 ± 1.8 $\mu\text{mol/L}$; NEFA, 591 ± 87 *v* 42 ± 16 $\mu\text{mol/L}$; both *P* < .001). Plasma glucose decreased at baseline after inhibition of lipolysis (Table 1). Plasma glucose decreased significantly during β_1 -adrenergic stimulation. This decrease was not affected by inhibition of lipolysis (-/BETA *v* no-LIP/BETA, 0.33 ± 0.04 *v* 0.33 ± 0.03 $\mu\text{mol/L}$). The baseline plasma insulin concentration was not affected by inhibition of lipolysis. Plasma insulin increased

significantly during β_1 -adrenergic stimulation alone (-/BETA), but did not change when lipolysis was inhibited (no-LIP/- and no-LIP/BETA). Plasma lactate concentrations were similar at baseline. Plasma lactate decreased significantly during β_1 -adrenergic stimulation without the preceding intervention (-/BETA). Plasma potassium levels were significantly different among trials at baseline and during infusion (Table 1). Post hoc testing showed no statistically significant differences between pairs of groups at baseline. Plasma potassium increased significantly after infusion of saline (no-LIP/-), but remained at similar levels during β_1 -adrenergic stimulation (-/BETA and no-LIP/BETA).

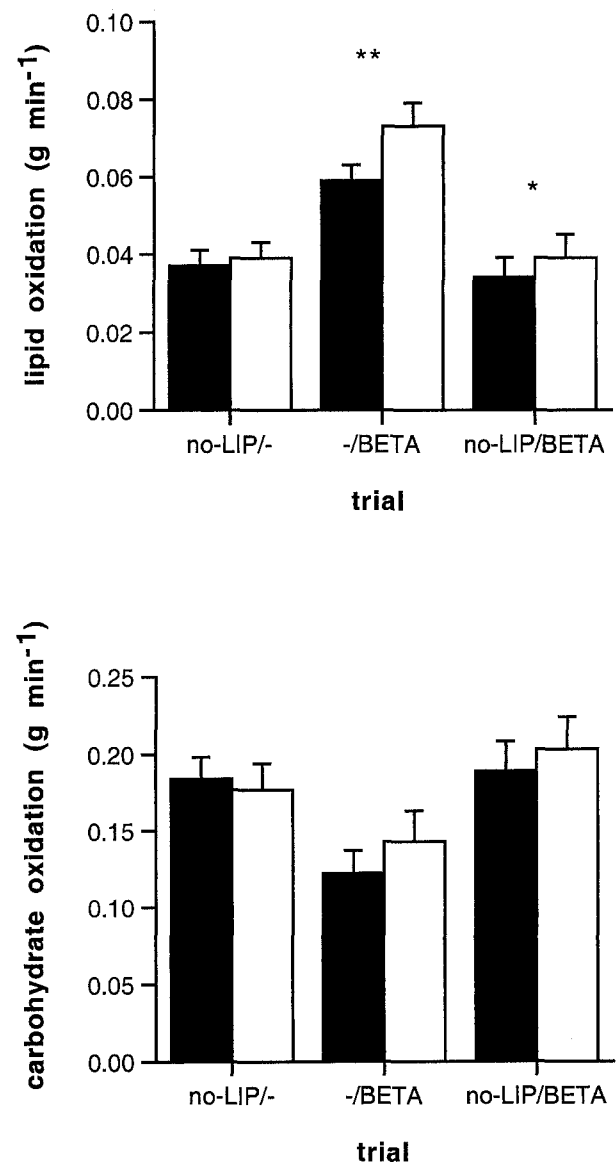


Fig 3. Lipid and carbohydrate oxidation (mean \pm SEM, n = 12) during baseline period (■) with or without inhibition of lipolysis (no-LIP) by acipimox and during infusion (□) of saline or dobutamine to stimulate β_1 -adrenoceptors (BETA). Student paired *t* test corrected for Bonferroni inequalities, baseline *v* infusion period: **P* < .05, ****P* < .01.

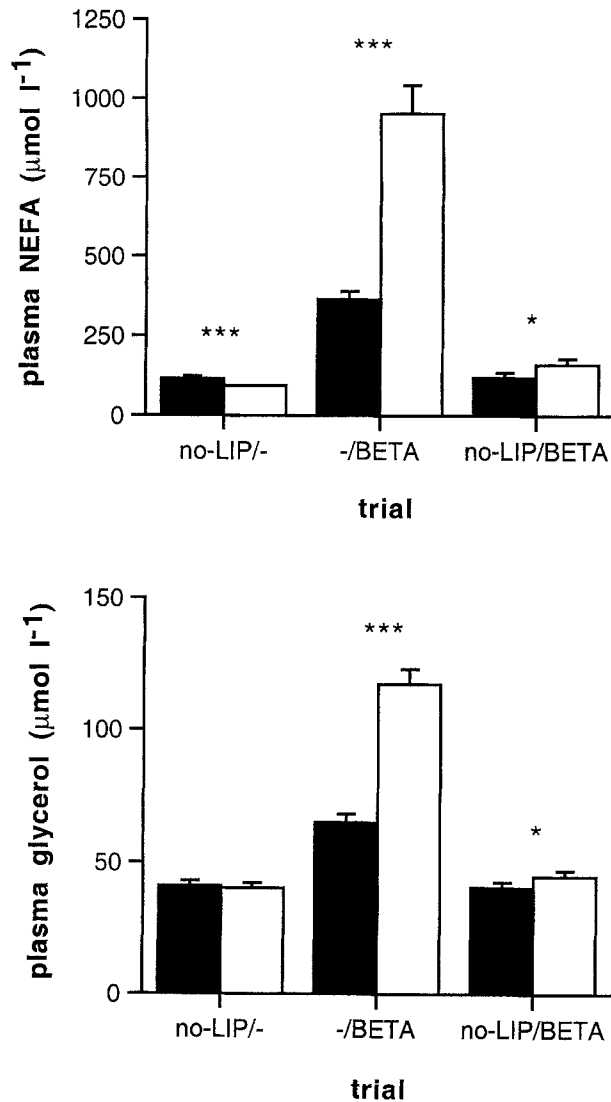


Fig 4. Plasma NEFA and glycerol concentrations (mean \pm SEM, $n = 12$) during baseline period (■) with or without inhibition of lipolysis (no-LIP) by acipimox and during infusion (□) of saline or dobutamine to stimulate β_1 -adrenoceptors (BETA). Student paired t test corrected for Bonferroni inequalities, baseline v infusion period: * $P < .01$, *** $P < .001$.

Heart Rate and Blood Pressure

The heart rate did not differ among trials at baseline, nor did it change during saline infusion or β_1 -adrenergic stimulation (baseline v infusion period: no-LIP/-, $58 \pm 3 v 58 \pm 3$ bpm; -/BETA, $56 \pm 3 v 57 \pm 2$; no-LIP/BETA, $55 \pm 3 v 56 \pm 2$). Baseline values for systolic and diastolic blood pressure were not different among trials (no-LIP/-, $119/80 \pm 2/2$ mm Hg; -/BETA, $122/76 \pm 2/2$; no-LIP/BETA, $120/80 \pm 2/2$). Saline infusion had no effect on systolic and diastolic blood pressure. Systolic blood pressure increased ($P < .001$) and diastolic blood pressure decreased ($P < .05$) to a similar extent during β_1 -adrenergic stimulation alone and β_1 -adrenergic stimulation with concomitant inhibition of lipolysis (-/BETA, $171/65 \pm 4/3$ mm Hg; no-LIP/BETA, $167/69 \pm 4/3$) (Fig 5).

Almost every subject showed an increase in energy expenditure, lipid oxidation, and lipolysis during β_1 -adrenergic stimulation with or without concomitant inhibition of lipolysis. Only two of 12 subjects showed no change in the lipid oxidation or lipolysis rate during the no-LIP/BETA trial. No subject responded in an opposite direction. Expression of the above-mentioned data per kilogram of fat-free mass did not change the interpretation of this study.

DISCUSSION

The present study was performed to investigate the mechanism underlying the increase in energy expenditure during β_1 -adrenergic stimulation. Lipolysis was inhibited by acipimox and β_1 -adrenoceptors were stimulated by infusion of dobutamine to determine whether a reduced availability of plasma NEFA would result in a reduced increase in energy expenditure and lipid oxidation.

Inhibition of lipolysis was accomplished by acipimox administration. Acipimox suppresses intracellular cyclic adenosine monophosphate levels in adipose tissue, which leads to a reduced hormone-sensitive lipase activity.¹⁷ In our study, plasma glycerol and NEFA concentrations decreased significantly after acipimox administration, indicating that a substantial blockade of adipose tissue lipolysis was achieved. After pretreatment with acipimox, glycerol and NEFA levels remained low during saline infusion (no-LIP/-), indicating that inhibition of lipolysis was present for the full 60 minutes of the trial. At baseline, the reduced availability of plasma NEFA was associated with a decrease in lipid oxidation (no-LIP/- and no-LIP/BETA), while energy expenditure was unchanged.

β_1 -Adrenergic stimulation with dobutamine significantly increased lipolysis; plasma glycerol increased 80% and plasma NEFA 163% (-/BETA). This is in accordance with prior studies by Green et al.¹¹ and Bhatt et al.¹⁰ Inhibition of lipolysis with acipimox, for the most part, prevented the increases in plasma glycerol and NEFA during β_1 -adrenergic stimulation, but plasma glycerol still increased 11% and plasma NEFA 36% (no-LIP/BETA). This indicates that lipolysis was not completely blocked by acipimox. Energy expenditure increased 19% during β_1 -adrenergic stimulation alone. This is also in accordance with the study by Green et al.,¹¹ which found an increase of 17%. Inhibition of lipolysis reduced the increase in energy expenditure to 8% during β_1 -adrenergic stimulation. Lipid oxidation increased 24% during β_1 -adrenergic stimulation alone. When lipolysis was inhibited, the increase in lipid oxidation during β_1 -adrenergic stimulation was attenuated, but it still increased 15%.

The interpretation of the findings of our study depends largely on the specificity of dobutamine as a β_1 -adrenoceptor agonist. Dobutamine predominantly stimulates β_1 -adrenoceptors, but also has some β_2 - and α_1 -adrenoceptor-stimulating properties.^{11,18} It could therefore directly stimulate skeletal muscle β_2 -adrenoceptors and thus bias our data. We checked for this confounder by determining plasma potassium concentrations. Potassium levels decrease during β_2 -adrenergic stimulation, but in our study, no changes in potassium concentrations were found during dobutamine infusion. This indicates that no significant β_2 -adrenergic stimulation occurred. This is in agree-

Table 1. Plasma Metabolite Concentrations During Baseline With or Without Inhibition of Lipolysis by Acipimox and During Infusion of Saline or Dobutamine to Stimulate β_1 Adrenoceptors

Metabolite	no-LIP/-		-/BETA		no-LIP/BETA		ANOVA	
	Baseline	Infusion	Baseline	Infusion	Baseline	Infusion	Baseline	Infusion
Glucose (mmol/L)	4.72 \pm 0.10	4.57 \pm 0.12	5.06 \pm 0.09	4.73 \pm 0.07‡	4.88 \pm 0.13	4.56 \pm 0.13‡	<i>P</i> < .001	NS
Insulin (mU/L)	5.33 \pm 0.48	4.97 \pm 0.50	5.94 \pm 0.67	7.66 \pm 0.67†§	5.44 \pm 0.48	5.78 \pm 0.54¶	NS	<i>P</i> < .01
Lactate (μ mol/L)	771 \pm 56	748 \pm 51	751 \pm 55	666 \pm 49†	782 \pm 41	772 \pm 40	NS	NS
Potassium (mmol/L)	4.16 \pm 0.08	4.26 \pm 0.09*	4.14 \pm 0.06	4.17 \pm 0.07	4.32 \pm 0.08	4.35 \pm 0.08#**	<i>P</i> < .05	<i>P</i> < .05

NOTE. Values are the mean \pm SEM for 12 subjects.

Student paired *t* test corrected for Bonferroni inequalities: within trials, baseline v infusion period, **P* < .05, †*P* < .01, ‡*P* < .001; between trials, -/BETA v no-LIP/-, §*P* < .05, ||*P* < .01; no-LIP/BETA v -/BETA, ¶*P* < .05, #*P* < .01; no-LIP/BETA v no-LIP/-, ***P* < .05.

ment with other studies indicating that β_2 -adrenoceptor-mediated effects of dobutamine are relatively small in relation to β_1 -adrenoceptor-mediated effects and become significant only at higher doses ($\geq 6 \mu\text{g}/\text{kg} \cdot \text{min}$).¹⁸ In a study by Green et al¹¹ (using the same dose of dobutamine as our study), no change in the plasma epinephrine concentration and a decrease in plasma norepinephrine was found during β_1 -adrenergic stimulation with dobutamine, indicating no additional stimulation from endogenous catecholamines. These findings suggest that the dose of dobutamine we used was specific for only β_1 -adrenergic stimulation.

It is suggested by several investigators^{8,19} that the catecholamine-induced increase in whole-body energy expenditure may be partly explained by the increase in myocardial energy expenditure caused by an increase in cardiac output. Myocardial energy expenditure can be estimated by the rate-pressure product (heart rate \times systolic blood pressure).²⁰ In our study, the estimated increase in myocardial energy expenditure during β_1 -adrenergic stimulation would result in an overall increase in energy expenditure of 5%. However, whole-body energy

expenditure increased 19% during β_1 -adrenergic stimulation alone and 8% during β_1 -adrenergic stimulation with concomitant inhibition of lipolysis. The majority of the increase in energy expenditure during dobutamine infusion alone therefore appears to result from substrate oxidation in other tissues.

Insulin is assumed to inhibit lipolysis and lipid oxidation. The increase in plasma insulin levels during β_1 -adrenergic stimulation alone would therefore tend to reduce the increase in lipolysis and lipid oxidation. However, when lipolysis was inhibited, plasma insulin levels remained low during β_1 -adrenergic stimulation and thus could not have caused the reduction in lipolysis and lipid oxidation compared with -/BETA.

Summarizing these results, the reduced availability of plasma NEFA after pretreatment with acipimox was accompanied by a reduced increase in energy expenditure and lipid oxidation during β_1 -adrenergic stimulation. This suggests that part of the dobutamine-induced increase in energy expenditure depends on NEFA availability. This may be the part that is localized in tissues without β_1 -adrenoceptors, such as skeletal muscle, in which energy expenditure cannot be directly increased by dobutamine. Whether an increased plasma NEFA concentration without β_1 -adrenergic stimulation can increase energy expenditure is still debated. After infusion of a lipid-heparin mixture that increases the plasma NEFA concentration, some studies found no change in energy expenditure²¹ or lipid oxidation,²² whereas others^{23,24} reported significant increases in energy expenditure. Our study is not able to answer the question as to whether the increased NEFA availability stimulates lipid oxidation, which in turn increases energy expenditure, or whether the increased NEFA availability stimulates energy expenditure, which is met by oxidation of more fat. The recent discovery of uncoupling proteins 2 and 3 in human skeletal muscle, which probably are upregulated by NEFA, would support the second possibility.²⁵

In conclusion, β_1 -adrenergic stimulation with dobutamine caused an increase in lipolysis, thermogenesis, and lipid oxidation. Simultaneous inhibition of lipolysis with acipimox was associated with a reduced increase in thermogenesis and lipid oxidation during β_1 -adrenergic stimulation.

ACKNOWLEDGMENT

The authors wish to thank Jos Stegen and Joan Senden for technical support during analysis of the blood samples.

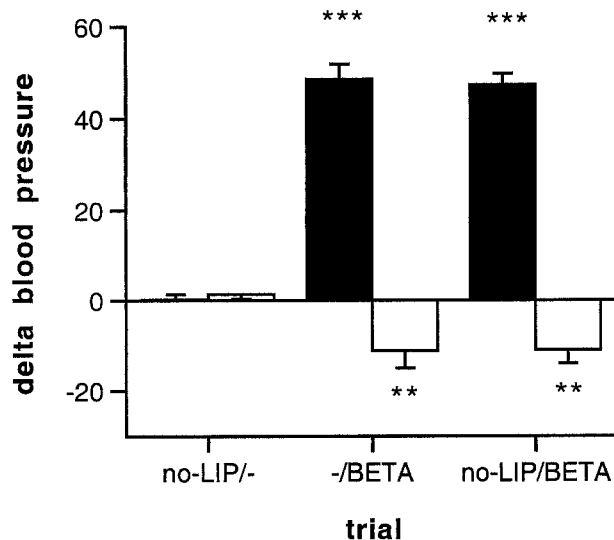


Fig 5. Changes in systolic (■) and diastolic (□) blood pressure (mean \pm SEM, *n* = 12) between baseline (with or without inhibition of lipolysis [no-LIP] by acipimox) and infusion period (saline or dobutamine to stimulate β_1 -adrenoceptors [BETA]). Student paired *t* test corrected for Bonferroni inequalities, baseline v infusion period: *P* < .01, ****P* < .001.**

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