Role of β -adrenoceptors in human obesity

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ROLE OF β -ADRENOCEPTORS IN HUMAN OBESITY

The Graduate School

nutrim

The studies presented in this thesis were performed at the Nutrition and Toxicology Research Institute Maastricht (NUTRIM) which participates in the graduate school VLAG (Food Technology, Agrobiotechnology, Nutrition and Health Sciences), accredited by the Royal Netherlands Academy of Arts and Sciences.

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Proefschrift

ter verkrijging van de graad van doctor
aan de Universiteit Maastricht
op gezag van de Rector Magnificus,
Prof dr AC Nieuwenhuijzen Kruseman,
volgens het besluit van het College van Decanen,
in het openbaar te verdedigen op
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door

Sandra Leonie Hendrika Schiffelers

geboren te Heerlen op 27 oktober 1970

Promotor

Prof dr ir WHM Saris

Co-promotor

Dr MA van Baak

Beoordelingscommissie

Prof dr H Kuipers (voorzitter)

Dr JRS Arch (Smithkline Beecham, United Kingdom)

Dr LMAB van Bortel

Prof dr HAJ Struijker Boudier

Prof dr J Zaagsma (Rijksuniversiteit Groningen)

*En als dat echte leven erbij in dreigt te schieten,
ga dan eens wandelen, het veld in
en kijk naar de vogels, de bloemen en het gras...*

Max van der Schoot
1925-1999

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INTRODUCTION 1

Epidemiology of obesity

Obesity can be defined as an excessive accumulation of body fat resulting in an increased body weight. The common unit to define obesity is the body mass index (BMI), which can be calculated by dividing a subject's weight in kilograms by the square of his height in meters. According to the World Health Organization-endorsed international classification, a BMI ≥ 30 kg/m² is associated with obesity.¹ Data from the MONICA-project (MONItoring project on CArdiovascular disease risk factors) collected in the period 1989-1996 show a mean prevalence of obesity in Europe of about 15% in men and 20% in women, aged 35-64 years, with the lowest prevalence in Belgium (men: 10%, women: 11%) and the highest prevalence in the Czech Republic (men: 22%, women: 29%).² In 1997, the prevalence of obesity in The Netherlands was 8% in men and 10% in women, aged 20-65 years.³ The variation in prevalence of obesity between countries might be explained by demographic factors (age, gender, ethnicity), socio-cultural differences (education level and income) and behavioral factors (dietary intake, smoking, alcohol consumption).^{1,4,5} Furthermore, there is a trend that the prevalence of obesity increases over time (figure 1.1).⁵

Figure 1.1 Prevalence of obesity (BMI ≥ 30 kg/m²) over time in men in England (●), Finland (Δ), Germany (◆) and The Netherlands (□) (adapted from Seidell *et al.*⁵).

Obesity is a major risk factor for many diseases which result from prosperity, such as coronary heart disease, hypertension, dyslipidemia and non-insulin dependent diabetes mellitus. A BMI ≥ 30 kg/m² is associated with a relative risk of 2.4 for coronary heart disease mortality and 9.5 for non-insulin dependent diabetes mellitus.⁵ However, a subsequent weight loss of 10% already significantly improves mortality of these diseases.^{1,6} Data from the Swedish Obese Subjects intervention study show that a decrease in body weight is associated with significant improvements in blood pressure and blood lipids, glucose and insulin concentrations (see figure 1.2).⁷ Weight loss might also prevent the onset of comorbidities. A study including 530 Chinese individuals with impaired glucose tolerance shows that after 6 years, the incidence of non-insulin dependent diabetes mellitus was ~ 43% in the weight-losing groups receiving dietary therapy, physical therapy or both, but ~ 68% in the non-treated control group.⁸

Weight loss therapy

Since weight loss is so effective in improving or preventing co-morbidities, an effective strategy for the treatment of obesity and more particular for the long-term management of weight control should be developed. Therefore, more knowledge is required on the regulatory mechanisms that control energy balance. To lose body weight, a negative energy balance is needed. This can be achieved by reducing energy intake, increasing energy expenditure or a combination of both. Consequently, the body will supplement the shortage of energy by burning its own energy (fat) reserves or tune down the energy needs of the metabolic active tissues. To maintain body weight or prevent body weight regain after a weight loss period, energy balance should be maintained. This implies that energy intake equals energy expenditure.⁹

The National Institutes of Health in the USA recommend a weight loss therapy which consists of dietary therapy (decreasing energy intake), increased physical activity (increasing energy expenditure) and behavioral therapy. This therapy should result in a decrease of 5-15% of the initial body weight at a rate of 0.5 kg per week.⁶ When the desired body weight is obtained, weight loss therapy is mostly considered to be ended. However, 40-60% of the weight loss is regained within 1 year and a complete return to the initial body weight tends to occur within 5 years.¹⁰ Maintenance of the lower body weight should therefore be added

Figure 1.2 Adjusted risk factor changes (%) in relation to body weight changes over 2 years in 842 men and women with obesity (adapted from Sjöström *et al.*⁷). BP: blood pressure.

as an additional goal to weight loss therapy. Especially continuing increased physical activity should be encouraged, since this significantly slows down the process of regaining body weight.¹¹

A combination therapy of a diet, exercise and behavioral treatment is preferred over pharmacologic therapy, since it changes patient's lifestyle and therefore its effects may be suitable for the rest of their life. However, support with pharmacologic agents may be a useful adjunct. At this moment, only two drugs, sibutramine and orlistat, are approved by the American Food and Drug Administration for use as anti-obesity drugs.

Sibutramine is a norepinephrine and serotonin reuptake inhibitor which increases satiety,¹² and thus decreases energy intake. Furthermore, it has a potential thermogenic effect which prevents the decline in energy expenditure that follows weight loss.^{12,13} In combination with a very-low-calorie diet (5 MJ/day), subjects receiving sibutramine (10 mg/day) for 12 weeks are found to lose 6.4% of their initial body weight as compared to 3.3% in the placebo group.¹⁴ In another study, all subjects received instructions to follow a very-low-calorie diet (5-6 MJ/day) at baseline, but no further counseling was given during the treatment period. After 24 weeks, subjects who additionally received sibutramine (10 mg/day) lost 6.1% of their initial body weight, whereas subjects receiving placebo lost 1.2% of their initial body weight.¹⁵ These studies show that sibutramine is a useful adjunct to promote weight loss during dietary therapy. In addition, sibutramine partly prevents weight regain after a period of weight loss. After a 4-week treatment period with a very-low-calorie diet, subjects randomly assigned to sibutramine (10 mg/day) lost a further 5.4% of their body weight attained after the diet, while patients in the placebo group regained 0.5%.¹⁶ Adverse effects of sibutramine are an increase in heart rate and blood pressure,¹²⁻¹⁶ which makes it unsuitable for overweight patients with concomitant cardiovascular disease or hypertension.

Orlistat inhibits the function of pancreatic lipase and prevents the absorption of ~ 30% of the dietary fat consumed, thus decreasing energy intake. Until now, two controlled studies are published which evaluate the efficacy of orlistat over a 2 year period. In the first year, orlistat (360 mg/day) was given in conjunction with a hypocaloric diet (3 MJ/day deficit). Mean weight loss over this year was 8.8%¹⁷ to 10.2%¹⁸ in the orlistat group and 5.8%¹⁷ to 6.1%¹⁸ in the placebo group. In the second year, all subjects which had received orlistat were placed on an eucaloric diet. Patients still receiving orlistat regained only half as much weight as compared to those who were switched to placebo.^{17,18}

After cessation of the medication, sibutramine or orlistat, all patients regained body weight independent of which medication they used. Patients that lost the most weight during the treatment phase regained the most weight after cessation.^{15,17,18} The results from these studies indicate, that anti-obesity drugs beneficially support the processes of weight reduction and weight maintenance. Therefore, pharmacologic treatment could be an important adjunct to the therapies of first choice.

Sympathetic nervous system

Thermogenesis

The sympathetic nervous system (SNS) plays an important role in the regulation of energy expenditure. Impaired SNS activity leads to a decrease in energy expenditure and may play a role in the etiology of obesity, whereas stimulation of the SNS leads to an increase in energy expenditure and may be a useful tool for the treatment and prevention of obesity. Therefore, it is certainly one of the first target physiological functions which has to be studied in relation to obesity.

SNS activity is stimulated in response to food digestion and physical exercise, but it can also be triggered by cold exposure or pathogenic stimuli.¹⁹ In response to these stimuli, sympathetic nerve fibers release norepinephrine into the synaptic cleft, where it functions as a neurotransmitter. The excess of norepinephrine spills over into the blood, where it acts as a hormone. Furthermore in response to these stimuli, the adrenal medulla excretes epinephrine into the blood. The SNS exerts its effects through norepinephrine and epinephrine binding to α_1 -, α_2 -, β_1 -, β_2 - and β_3 -adrenoceptors on target cells. Table 1.1 gives an overview of the localization of these adrenoceptor subtypes and the effects they mediate.²⁰

During the infusion of norepinephrine,^{21,22} epinephrine^{23,24} or isoprenaline²⁵ (non-selective β -adrenoceptor agonist), thermogenesis increases significantly. The role of the individual adrenoceptor subtypes in thermogenesis is not completely known. α -Adrenergic stimulation does not affect whole body thermogenesis,²⁵⁻²⁸ whereas only β_1 -^{29,30} or only β_2 -adrenergic stimulation^{25,29,31} increases thermogenesis. The role of the β_3 -adrenoceptor in human energy and substrate metabolism is still debated,^{25,32,33} since no specific full β_3 -adrenoceptor agonist or antagonist is available for administration in humans.

In obese subjects, the increase in energy expenditure has been found to be impaired during norepinephrine³⁴ or isoprenaline³⁵ infusion, although others found no decreased norepinephrine,²⁴ epinephrine³⁶ or isoprenaline-induced³¹ thermogenic response. Assuming that energy intake in subjects at risk for obesity is similar to that in subjects who are not at risk, but their thermogenic response is impaired due to decreased SNS activity, these subjects will be more prone to a positive energy balance and weight gain.

Table 1.1 Effects caused by stimulation of the different adrenoceptor subtypes in humans.

Tissue	Effect	Adrenoceptor subtype				
		α_1	α_2	β_1	β_2	β_3
Heart	Rate			Increase		
	Force of contraction			Increase		
Skeletal muscle	Tremor				Increase	
Adipose tissue	Lipolysis		Decrease	Increase	Increase	Increase?
Liver	Glycogenolysis	Decrease			Increase	
Bronchi		Constrict			Dilate	
Blood vessels		Constrict	Constrict		Dilate	
Pancreas	Insulin secretion		Decrease		Increase	

Lipid utilization

The relatively high-fat diets consumed nowadays have been suggested to be an important factor in the etiology of obesity. Since the increase in fat consumption coincided with a decrease in carbohydrate consumption³⁷ and fat has a higher energy density than carbohydrates (37 kJ/g vs 16 kJ/g), this could easily have resulted in an increased energy intake. Furthermore, carbohydrate balance is accurately regulated through automatic increases in carbohydrate oxidation in response to excess intake, whereas in the case of fat there is virtually no auto-regulatory linkage which would act to maintain fat balance^{38,39} and weight gain occurs by excess intake.

The SNS also plays an important role in stimulating lipolysis and lipid oxidation. During the infusion of norepinephrine,^{21,22} epinephrine^{23,24} or isoprenaline,³¹ lipolysis and lipid oxidation significantly increase in lean subjects. However, these increases appear to be impaired in obese subjects during epinephrine^{24,40} or isoprenaline³¹ infusion, although this finding is not consistent.⁴¹ Assuming that lipolysis is impaired in obese subjects, less triglycerides are hydrolyzed to glycerol and non-esterified fatty acids (NEFA) in the adipose tissue, therefore less NEFA become available in the blood stream and thus less NEFA can be oxidized in skeletal muscle, where lipid oxidation is presumed to be predominantly localized (figure 1.3).^{31,42} If lipid oxidation is impaired as well, only part of the available NEFA will be oxidized and the remaining part has to be stored again. These presumed impaired responses in lipolysis and lipid oxidation may play a role in the development or maintenance of relatively increased fat stores.

Obesity

The question remains whether impaired SNS activity is a cause or a consequence of obesity. Blaak *et al.*⁴³ showed that isoprenaline-induced thermogenesis tended to increase after weight loss. This improvement in β -adrenoceptor-mediated thermogenesis after weight loss suggests that the impaired SNS response is a consequence of the obese state. On the other hand, Astrup *et al.*⁴⁴ showed that glucose-induced increases in energy expenditure and norepinephrine levels improved in obese subjects after 30 kg weight loss, but were still lower than that in control subjects. Furthermore, Blaak *et al.*⁴³ showed that isoprenaline-induced increases in arterial NEFA concentration, as indicator for lipolysis, and muscle NEFA uptake, as indicator for lipid oxidation, remained impaired after weight reduction. This suggests that a defective SNS may rather be a primary factor leading to the development of obesity than a secondary factor resulting from the obese state.

Body weight loss during a very-low-calorie diet comprises of 80% fat mass loss and 20% fat free mass loss.^{43,44} Moreover, high pretreatment plasma norepinephrine levels, suggesting high SNS activity, have been shown to be associated with a better weight loss outcome in women.⁴⁵ This suggests that stimulation of the SNS may play an important role in the treatment of obesity. In rats, β_3 -adrenergic stimulation leads to significant increases in thermogenesis and lipid utilization which subsequently results in weight loss.^{46,47} If this is also the case in humans, specific β_3 -adrenoceptor agonists may be used as potential anti-obesity drugs, especially since they cause no β_1 -adrenoceptor-mediated tachycardia or β_2 -adrenoceptor-mediated tremor. However, the human β_3 -adrenoceptor differs pharmacologically from the rat β_3 -adrenoceptor.^{48,49} Consequently, the β_3 -adrenoceptor agonists used

Figure 1.3 The role of the individual β -adrenoceptor subtypes in thermogenesis and lipid utilization.

in rats are only weak agonists in humans. Until now, no specific full β_3 -adrenoceptor agonist is available for administration in humans. Administration of the relatively unselective β_3 -adrenoceptor agonist BRL 26830A for 18 weeks has been shown to be successful in promoting additional weight loss during a very-low-calorie diet compared to placebo.⁵⁰ Two weeks of BRL 35135 administration improved glucose tolerance, but did not change body weight in obese subjects on a weight-maintaining diet.⁵¹ Unfortunately due to their relative unselectivity, adverse effects like tremor and increases in heart rate and blood pressure occurred which made them unsuitable for long-term usage.

In conclusion, the SNS plays an important role in energy and substrate metabolism. The impaired response on SNS activity in obese subjects may play a role in the development or maintenance of the obese state. SNS stimulation is therefore a target for anti-obesity therapy.

Outline of the thesis

The research presented in this thesis focusses on the role of the different β -adrenoceptor subtypes of the SNS in the regulation of thermogenesis and lipid utilization in human obesity.

Role of β -adrenoceptor subtypes

In order to study the effect of only β_1 -, only β_2 - or only β_3 -adrenergic stimulation, selective β -adrenoceptor agonists are needed. However, most available β -adrenoceptor agonists lose their specificity for a certain adrenoceptor subtype as the dose of administration increases. Therefore, we investigated up to which dosage dobutamine can be used as selective β_1 -

adrenoceptor agonist and salbutamol can be used as selective β_2 -adrenoceptor agonist (chapter 2). At this moment, no selective β_3 -adrenoceptor agonist is available for administration in humans. The non-selective β -adrenoceptor agonist isoprenaline might stimulate β_3 -adrenoceptors at an ~ 100-fold higher dosage as it stimulates β_1 - and β_2 -adrenoceptors, as was shown in *in vitro* studies with isolated human fat cells.^{52,53} *In vivo* studies, however, show contradictory results on the ability of isoprenaline to stimulate the human β_3 -adrenoceptor.^{31,33} We studied whether a high dosage of isoprenaline may be used as selective β_3 -adrenoceptor agonist when it is given in combination with the β_1 - and β_2 -adrenoceptor antagonist nadolol or propranolol (chapter 3).

NEFA availability

Stimulation of β_1 -adrenoceptors with dobutamine causes significant increases in lipolysis, lipid oxidation and thermogenesis.^{30,54} The rise in lipolysis can be explained by the presence of β_1 -adrenoceptors in adipose tissue. The rise in lipid oxidation and energy expenditure is assumed to be localized predominantly in skeletal muscle,^{31,42} but this tissue contains mainly β_2 -adrenoceptors and presumably no β_1 -adrenoceptors.⁵⁵ Therefore, β_1 -adrenergic stimulation is not likely to increase energy expenditure and lipid oxidation by direct stimulation of skeletal muscle (figure 1.3). Indirectly as a secondary effect of β_1 -adrenergic stimulation, the elevated levels of NEFA in blood, caused by increased lipolysis, could have induced the observed increases in lipid oxidation and energy expenditure. We tested this hypothesis by inhibiting lipolysis with acipimox and subsequently stimulating β_1 -adrenoceptors with dobutamine to measure the remaining increases in lipid oxidation and thermogenesis (chapter 4).

Obesity

SNS stimulation leads to impaired responses in thermogenesis^{34,35} and lipid utilization^{24,31,40} in obese subjects. Until now, it is unclear which β -adrenoceptor subtype is responsible for these impaired responses. *In vitro* studies with isolated human fat cells suggest a role for the β_2 -adrenoceptor, since β_1 -adrenoceptor-mediated lipolysis is similar in obese and lean subjects, but β_2 -adrenoceptor-mediated lipolysis is impaired in the obese.⁵⁶ We studied the *in vivo* effects of β_1 - and β_2 -adrenergic stimulation on thermogenesis and lipid utilization in obese and lean men (chapter 5).

If the availability of NEFA in blood is a limiting factor for thermogenesis, the reduced lipolytic response in obese subjects during sympathetic stimulation might explain their reduced response in lipid oxidation and thermogenesis. On the other hand, Colberg *et al.*⁵⁷ and Simoneau *et al.*⁵⁸ found that obese women have a decreased oxidative capacity and increased glycolytic and anaerobic capacities, as measured by the activity of several key enzymes in skeletal muscle biopsies. This suggests that obese subjects favor carbohydrates above lipids as a fuel and store the available NEFA as fat. To examine whether the impaired response in thermogenesis and lipid oxidation in the obese is caused by a decreased NEFA availability, we increased plasma NEFA concentrations in obese and lean men by infusing a lipid heparin mixture and measured the consequent increases in lipid oxidation and energy expenditure (chapter 6).

Patients with chronic obstructive pulmonary disease (COPD) commonly have a relatively increased fat mass which is, however, not associated with overweight. In contrast, weight loss is often seen in these patients. Although normal weight loss merely comprises loss of fat and fat free mass, patients with COPD may show a depletion of fat free mass despite a relative preservation of fat mass.^{59,60} In the latter group, functional capacity characterized by decreased muscle function, exercise capacity and even health status is more impaired as compared to underweight subjects with a normal fat free mass.⁶¹ Furthermore, recent studies indicate that the relative to absolute increase in fat mass and decrease in fat free mass in COPD patients might be related to intrinsic deviations in substrate metabolism.⁶²⁻⁶⁴ We investigated whether development or maintenance of a relatively increased fat mass in normal weight patients with COPD, despite periods of weight loss, is related to an impaired β -adrenergic response in lipid utilization and thermogenesis, as seen in obese subjects (chapter 7).

Finally, the results from the different studies are summarized and discussed in the general discussion (chapter 8).

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**DOBUTAMINE AS SELECTIVE
 β_1 -ADRENOCEPTOR AGONIST IN
IN VIVO STUDIES ON
HUMAN THERMOGENESIS
AND LIPID UTILIZATION**

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SLH Schiffelers, VJA van Harmelen, HAJ de Grauw,
WHM Saris and MA van Baak

Nutrition Toxicology and Environment Research Institute Maastricht,
Department of Human Biology, Maastricht University,
Maastricht, The Netherlands

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Abstract

Aim: To investigate whether dobutamine can be used as a selective β_1 -adrenoceptor agonist in *in vivo* studies on human thermogenesis and lipid utilization.

Subjects: Twenty healthy males with a mean body mass index of 21.9 kg/m^2 (range: $19.4\text{--}25.3 \text{ kg/m}^2$) and a mean age of 22 y (range: 18–27 y).

Design: In the *dobutamine test*, a 30 min baseline period was followed by consecutive infusions of 2.5, 5 and $10 \text{ }\mu\text{g/kg.min}$ dobutamine (selective β_1 -adrenoceptor agonist), each dose during 30 min. In the *dobutamine plus atenolol test*, dobutamine was given (as described above) in combination with the β_1 -adrenoceptor antagonist atenolol (prime: $42.5 \text{ }\mu\text{g/kg}$, infusion: $1.02 \text{ }\mu\text{g/kg.min}$). The *saline test* consisted of a 30 min baseline period followed by three times a 30 min period of saline infusion. The *salbutamol plus atenolol test* consisted of a 45 min baseline period, after which 85 ng/kg.min salbutamol (selective β_2 -adrenoceptor agonist) was given for 90 min. During the last 45 min, atenolol was added to the salbutamol infusion at the same dose, as described above.

Measurements: Energy expenditure and respiratory exchange ratio, as indicator for lipid oxidation, were measured by indirect calorimetry. At the end of each infusion period, a blood sample was taken for the determination of plasma non-esterified fatty acids and glycerol concentrations, as indicators for lipolysis.

Results: Dobutamine induced significant increases in energy expenditure, lipid oxidation and lipolysis. The β_1 -adrenoceptor antagonist atenolol blocked all dobutamine-induced effects on thermogenesis and lipid utilization. All parameters remained at levels comparable with those during saline infusion. The used dosage of atenolol did not inhibit β_2 -adrenoceptor-specific changes in energy expenditure, lipid oxidation and lipolysis during salbutamol infusion. This indicates that atenolol was specific for β_1 -adrenoceptors and did not camouflage concomitant β_2 -adrenoceptor stimulation during dobutamine infusion.

Conclusion: Dobutamine can be used as a selective β_1 -adrenoceptor agonist at dosages $\leq 10 \text{ }\mu\text{g/kg.min}$ in *in vivo* studies on human thermogenesis and lipid utilization.

Introduction

The sympathetic nervous system plays an important role in the regulation of human thermogenesis. Sympathetic nervous system activity is mainly stimulated in response to food digestion and physical exercise, but can also be triggered by cold exposure or pathogenic stimuli. In response to these stimuli, catecholamines are released which subsequently induce thermogenesis.¹ This increase in energy expenditure is due to stimulation of both β_1 - and β_2 -adrenoceptors of the sympathetic nervous system.² α -Adrenoceptors probably do not play a role.²⁻⁴ The effect of β_3 -adrenergic stimulation on human thermogenesis is still debated at this moment,^{2,5,6} since the available agonists appear to be only weak partial agonists in humans.⁷ In rodents, β_3 -agonists induce significant effects, but this might be explained by the pharmacological differences between human and rodent β_3 -adrenoceptors.^{8,9}

In obese men, non-selective β -adrenergic stimulation leads to a reduced increase in thermogenesis and lipid utilization compared to lean men.¹⁰ Therefore, it is interesting to examine whether these impaired responses might be due to a defect in the β_1 - or the β_2 -adrenoceptor. The most selective β_1 -adrenoceptor agonist for *in vivo* use in humans is dobutamine. In healthy volunteers, dobutamine increases oxygen consumption, indicating an increase in thermogenesis,^{11,12} decreases the respiratory exchange ratio (RER), indicating an increase in lipid oxidation, and increases plasma glycerol and non-esterified fatty acids (NEFA) concentrations, indicating an increase in lipolysis.¹¹

However, both *in vitro*^{9,13} and *in vivo*^{14,15} animal studies have shown that dobutamine also has α_1 - and β_2 -adrenoceptor agonistic properties. Since α_1 -adrenoceptors are not important for human thermogenesis, their role was not further investigated. The selectivity of dobutamine for β_1 - and β_2 -adrenoceptors in studies on human thermogenesis and lipid utilization was elucidated in this study. Therefore, we evaluated the effect of atenolol, a predominantly β_1 -adrenoceptor antagonist, on dobutamine-induced increases in energy expenditure, lipid oxidation and lipolysis. Addition of atenolol should block all β_1 -adrenoceptor-mediated effects and reveal all other effects of dobutamine. In a control test, the selective β_1 -adrenoceptor-blocking properties of atenolol at the dose used were verified. Addition of atenolol should have no effect on the increases in thermogenesis and lipid oxidation induced by the selective β_2 -adrenoceptor agonist salbutamol.

Subjects and Methods

Subjects

Twenty lean male volunteers participated in this study. Mean body mass index and age were 21.9 kg/m² (range: 19.4-25.3 kg/m²) and 22 y (range: 18-27 y) respectively. The subjects were healthy and took no medication at the time of the study. They gave written informed consent before participating in the study. The study protocol was approved by the Ethics Committee of Maastricht University.

Experimental design

The study protocol consisted of four tests. In the *dobutamine test*, a 30 min baseline period was followed by consecutive infusions of 2.5, 5 and 10 $\mu\text{g}/\text{kg}\cdot\text{min}$ dobutamine (selective β_1 -adrenoceptor agonist) (Dobax,[®] Byk, Zwanenburg, The Netherlands), each dose during 30 min. This test intended to measure all dobutamine-mediated effects. The *saline test* consisted of a 30 min baseline period, followed by three times a 30 min period of saline infusion (0.6 ml/min) to study the regular changes in thermogenesis and associated metabolic processes over this period of fasting. In the *dobutamine plus atenolol test*, dobutamine was given (as described above) in combination with the β_1 -adrenoceptor antagonist atenolol (Tenormin,[®] Zeneca, Ridderkerk, The Netherlands) to reveal possible β_2 -adrenoceptor-mediated effects of dobutamine. Therefore, a priming dose of 42.5 $\mu\text{g}/\text{kg}$ atenolol was administered intravenously within 5 min at the start of the baseline period, after which a continuous infusion of atenolol (1.02 $\mu\text{g}/\text{kg}\cdot\text{min}$) was started for the remainder of the test. The *salbutamol plus atenolol test* consisted of a 45 min baseline period, after which the β_2 -adrenoceptor agonist salbutamol (Ventolin,[®] GlaxoWellcome, Zeist, The Netherlands) was given for 90 min at an infusion rate of 85 ng/kg.min. During the last 45 min, atenolol was added to the salbutamol infusion (at the same dose as described above) to study possible β_2 -adrenoceptor-blocking effects of atenolol. The infusion periods were prolonged during the last test, since thermogenesis did not reach steady state within 30 min during salbutamol infusion, as it did during dobutamine infusion. Twenty subjects participated in the dobutamine test, 10 subjects in the saline test, 14 subjects in the dobutamine plus atenolol test and 10 subjects underwent the salbutamol plus atenolol test. Each of the 20 subjects participated in two or three trials. There were no statistically significant differences in subject parameters between tests. The study design was single-blind and the order of tests was randomized.

The subjects came to the laboratory at 8:30 AM with at least two days between tests. All individuals were fasted for at least 10 hours (overnight) and came to the laboratory by car or by bus to minimize the amount of physical activity prior to the tests. At the beginning of each test, a catheter was inserted into a forearm vein for drug infusion and blood sampling. During the tests, energy expenditure and RER were continuously measured and at the end of each 30 or 45 min interval, a blood sample was obtained. For safety reasons, the infusion was stopped when heart rate had increased more than 30 beats/min and/or mean blood pressure had risen more than 30 mmHg. Following these criteria, one subject was not tested at the highest dose of 10 $\mu\text{g}/\text{kg}\cdot\text{min}$ dobutamine. Room temperature was kept between 23–25 °C.

Clinical methods

An open-circuit ventilated hood system was used for measurement of whole body energy expenditure and RER every 2 min. The volume of air drawn through the hood was measured by a dry-gas meter (Schlumberger, Dordrecht, The Netherlands) and the composition of the inflowing and outflowing air was analyzed by a paramagnetic O₂ analyzer (Servomex, Crowborough, UK) and an infrared CO₂ analyzer (Hartmann and Braun, Frankfurt, Germany). Airflow rate and the O₂ and CO₂ concentrations of the ingoing and outgoing air were used to compute O₂ consumption (coefficient of variation (CV): 2.4%) and CO₂ production (CV:

3.1%) on-line through an automatic acquisition system interfaced with a personal computer. Energy expenditure was calculated according to the formula of Weir.¹⁶ Energy expenditure and RER values were averaged over the last 10 min of each infusion step, during which their values were stable, and their means were used in the data analysis.

Blood pressure was measured by an automated blood pressure device (Tonoprint, Speidel & Keller, Jungingen, Germany) during the last 10 min of each period. The mean of four measurements per interval was computed and used for further analysis. Heart rate was monitored continuously by conventional electrocardiography and was recorded at the end of every 5 min period. The values over the last 10 min were averaged and used for further analysis.

Analytical methods

Blood samples for glycerol and NEFA determination were preserved in sodium-EDTA. All samples were immediately centrifuged for 1 min at 7000 $\times g$. Plasma was transferred to microtest tubes, rapidly frozen in liquid nitrogen and stored at -70°C until further analysis. Plasma glycerol concentrations were measured with a glycerol kit (148270, Boehringer, Mannheim, Germany) and plasma NEFA concentrations were measured with the NEFA C kit (99475409, WAKO, Neuss, Germany), both on a Cobas-Fara analyzer (Roche Diagnostica, Basel, Switzerland). In each run, standard samples with known concentrations were included for quality control.

Data analysis

All values are presented as mean $\pm$ standard error of the mean (SEM). The differences in outcome between the dobutamine, the saline and the dobutamine plus atenolol test were analyzed with a split-block incomplete block factorial ANOVA. In this design, categories are made for treatment and subject to account for missing values. Post hoc testing between

Figure 2.1 Energy expenditure and respiratory exchange ratio during infusion of saline (●) ($n = 10$ subjects), dobutamine (Δ) ($n = 20$ subjects) or dobutamine plus atenolol (□) ($n = 14$ subjects). Values are mean $\pm$ SEM. One-way repeated measurements ANOVA: ** $P < 0.01$, *** $P < 0.001$. Unpaired t-test corrected for Bonferroni's inequalities: dobutamine vs saline: * $P < 0.05$, *** $P < 0.001$; dobutamine vs dobutamine plus atenolol: ** $P < 0.01$, *** $P < 0.001$.

studies was done with an unpaired t-test, corrected according to Bonferroni's inequalities. The effects within studies were analyzed with one-way repeated measurements ANOVA. Post hoc testing between time points was done with a paired t-test, corrected according to Bonferroni's inequalities. All statistical tests were performed two-sided. A P-value < 0.05 was regarded as statistically significant.

Results

Dobutamine, saline and dobutamine plus atenolol tests

Energy expenditure increased significantly during dobutamine infusion ($P < 0.001$) (figure 2.1). During saline infusion, there was no significant change in energy expenditure. Simultaneous administration of atenolol completely prevented the dobutamine-induced increase in energy expenditure. It remained at a similar level as during the saline test. RER decreased significantly in all tests (dobutamine, saline: $P < 0.001$; dobutamine plus atenolol: $P < 0.01$) (figure 2.1). During the second and third infusion period of dobutamine, RER was significantly lower than during the corresponding infusion periods with saline (both $P < 0.001$). Atenolol prevented the more pronounced reduction of RER at the higher dobutamine dosages. RER decreased to a comparable level as during saline infusion.

Plasma glycerol and NEFA concentrations increased significantly with dobutamine (both $P < 0.001$), saline (glycerol: $P < 0.01$, NEFA: $P < 0.001$) as well as dobutamine plus atenolol (glycerol: $P < 0.01$, NEFA: $P < 0.001$) (figure 2.2). In all infusion periods, glycerol and NEFA concentrations were significantly higher in the dobutamine test compared to the saline and dobutamine plus atenolol test. During atenolol infusion, glycerol and NEFA levels remained similar to those during saline infusion.

During the last infusion period, heart rate was significantly higher with dobutamine than with saline ($P < 0.001$) (figure 2.3). Heart rate did not change during saline infusion.

Figure 2.2 Plasma glycerol and non-esterified fatty acids (NEFA) concentration during infusion of saline (●) ($n = 10$ subjects), dobutamine (Δ) ($n = 20$ subjects) or dobutamine plus atenolol (□) ($n = 14$ subjects). Values are mean $\pm$ SEM. One-way repeated measurements ANOVA: ** $P < 0.01$, *** $P < 0.001$. Unpaired t-test corrected for Bonferroni's inequalities: dobutamine vs saline: * $P < 0.05$, *** $P < 0.001$; dobutamine vs dobutamine plus atenolol: ** $P < 0.01$, *** $P < 0.001$.

Figure 2.3 Heart rate and systolic and diastolic blood pressure during infusion of saline (●) ($n = 10$ subjects), dobutamine (Δ) ($n = 20$ subjects) or dobutamine plus atenolol (□) ($n = 14$ subjects). Values are mean $\pm$ SEM. One-way repeated measurements ANOVA: * $P < 0.05$, *** $P < 0.001$. Unpaired t-test corrected for Bonferroni's inequalities: dobutamine vs saline: *** $P < 0.001$; dobutamine vs dobutamine plus atenolol: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; saline vs dobutamine plus atenolol: @ $P < 0.05$.

Atenolol lowered heart rate at baseline and completely inhibited the increase in heart rate by dobutamine. Systolic blood pressure increased significantly ($P < 0.001$) and diastolic blood pressure remained unchanged in the dobutamine test (figure 2.3). Saline infusion caused no changes in systolic and diastolic blood pressure. Atenolol inhibited most of the increase in systolic blood pressure, but there was still a significant increase ($P < 0.001$). Diastolic blood pressure did not change during dobutamine plus atenolol infusion.

Salbutamol test

Energy expenditure significantly increased during salbutamol infusion ($P < 0.001$) and remained at this level after the addition of atenolol (table 2.1). RER remained unchanged during the whole test. Plasma glycerol and NEFA concentrations increased significantly during salbutamol infusion (glycerol: $P < 0.01$, NEFA: $P < 0.001$) and decreased after the addition of atenolol (glycerol: $P < 0.05$, NEFA: $P < 0.001$). However, plasma glycerol and NEFA levels remained significantly higher with salbutamol plus atenolol compared to baseline (both $P < 0.01$). Heart rate and systolic blood pressure increased significantly with salbutamol (both $P < 0.001$) and decreased significantly after the addition of atenolol (heart rate: $P < 0.001$, systolic blood pressure: $P < 0.01$). Heart rate remained significantly higher during salbutamol plus atenolol infusion compared to baseline ($P < 0.001$), but systolic blood pressure did not differ from baseline during salbutamol plus atenolol administration. Diastolic blood pressure did not change during the test.

Table 2.1 Parameters at baseline, during salbutamol (85 ng/kg.min) infusion and during salbutamol plus atenolol (bolus: 42.5 µg/kg, infusion: 1.02 µg/kg.min) infusion.

Parameter	Baseline	Salbutamol	Salbutamol plus atenolol	ANOVA
Energy expenditure (kJ/min)	5.40 ± 0.19	6.20 ± 0.14 ^b	5.95 ± 0.20 ^f	P<0.001
Respiratory exchange ratio	0.83 ± 0.01	0.82 ± 0.01	0.83 ± 0.01	NS
Glycerol (µmol/l)	53.6 ± 4.7	115.2 ± 14.2 ^a	96.7 ± 13.1 ^{cg}	P<0.001
NEFA (µmol/l)	249 ± 32	710 ± 75 ^b	472 ± 65 ^{eg}	P<0.001
Heart rate (beats/min)	56 ± 2	70 ± 3 ^b	65 ± 3 ^{eh}	P<0.001
Systolic blood pressure (mmHg)	117 ± 3	130 ± 3 ^b	121 ± 4 ^d	P<0.001
Diastolic blood pressure (mmHg)	67 ± 2	61 ± 2	65 ± 3	P<0.05

Values are mean ± SEM for 10 subjects. NEFA: non-esterified fatty acids. Paired t-test corrected for Bonferroni's inequalities: rest vs salbutamol: ^aP<0.01, ^bP<0.001; salbutamol vs salbutamol plus atenolol: ^cP<0.05, ^dP<0.01, ^eP<0.001; rest vs salbutamol plus atenolol: ^fP<0.05, ^gP<0.01, ^hP<0.001.

Discussion

This study was performed to examine whether dobutamine can be used as a selective β_1 -adrenoceptor agonist in *in vivo* studies on human thermogenesis and lipid utilization. Dobutamine induced significant increases in energy expenditure, lipid oxidation, as measured by a decrease in RER, and lipolysis, as measured by increases in plasma glycerol and NEFA levels. This is in accordance with previous studies.^{11,12}

From animal studies, it is known that dobutamine has significant α_1 - and β_2 -adrenoceptor-stimulating properties at higher dosages *in vitro*.^{9,13-15} Furthermore, Daul *et al.*¹⁷ showed that these adrenoceptors also play a role in the regulation of dobutamine-induced changes in heart rate and blood pressure at dosages ≥ 6 µg/kg.min *in vivo* in humans. We intended to study the β_1 -adrenoceptor selectivity of dobutamine for changes in thermogenesis and lipid utilization. For that reason, β_1 -adrenoceptor-mediated effects of dobutamine on energy expenditure, lipid oxidation and lipolysis were blocked with the selective β_1 -adrenoceptor antagonist atenolol. This design should reveal all β_2 -adrenoceptor-mediated effects of dobutamine, since α -adrenoceptors play no role in human thermogenesis.²⁻⁴ We found that atenolol completely inhibited the dobutamine-induced increases in energy expenditure and plasma glycerol and NEFA concentrations and decrease in RER. These parameters remained at levels comparable with those during the saline test, suggesting that dobutamine affects these parameters only via β_1 -adrenoceptor stimulation at the dosages used.

A control test was done to evaluate the selectivity of the used dose of atenolol for β_1 - and β_2 -adrenoceptors. β_2 -Adrenoceptor-mediated effects of salbutamol were compared with those during simultaneous salbutamol plus atenolol infusion. If atenolol blocks β_2 -adrenoceptors, all responses on salbutamol infusion should be impaired after the addition of atenolol. We found that the salbutamol-induced change in energy expenditure was not affected by atenolol. Thus, it is rather unlikely that the diminished increase in thermogenesis during simultaneous dobutamine plus atenolol infusion was due to β_2 -adrenoceptor blockade

by atenolol at the dosage used. This is also supported by the fact that atenolol has a K_i of 72 ng/ml for β_1 -adrenoceptors and a K_i of 2519 ng/ml for β_2 -adrenoceptors.¹⁸ Thorne and Wahren¹⁹ reported a plasma atenolol concentration of about 300 ng/ml at a dose of 1.67 μ g/kg.min. This is comparable with a plasma concentration of about 180 ng/ml for the dose of atenolol we used (1.02 μ g/kg.min). The affinity of salbutamol for β_2 - and β_1 -adrenoceptors lies only 8-fold apart.²⁰ This suggests that concomitant β_1 -adrenoceptor stimulation during salbutamol infusion is more likely to have occurred than β_2 -adrenoceptor blockade during simultaneous atenolol infusion. The significant decreases in plasma glycerol and NEFA concentrations after the addition of atenolol might therefore be due to the blockade of the β_1 -adrenoceptor-mediated effects of salbutamol. Another explanation might be that atenolol blocked the basal β_1 -adrenoceptor-mediated effects of the endogenous catecholamines on lipolysis.

It is still uncertain which processes are responsible for sympathetically-mediated thermogenesis and in which tissues these processes are localized. Several authors^{21,22} have suggested that the catecholamine-induced increase in whole body energy expenditure may partly be 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).²³ In our study, the estimated increase in myocardial energy expenditure would result in an overall increase in energy expenditure of 14% during the dobutamine test and of 2% during the dobutamine plus atenolol test. Whole body energy expenditure, however, increased with 33% during the dobutamine test and with 5% during the dobutamine plus atenolol test. The majority of the increase in energy expenditure therefore appeared to result from substrate oxidation in other tissues.

In summary, the results of this study indicate that at dosages of 2.5, 5 and 10 μ g/kg.min the predominantly β_1 -adrenoceptor agonist dobutamine caused significant increases in energy expenditure, lipid oxidation and lipolysis. The β_1 -adrenoceptor antagonist atenolol blocked all dobutamine-induced increases in thermogenesis and lipid utilization. All parameters remained at levels comparable with those during saline infusion. The used dose of atenolol was specific for β_1 -adrenergic blockade and therefore did not camouflage concomitant β_2 -adrenoceptor stimulation by dobutamine. Therefore, we conclude that dobutamine can be used as selective β_1 -adrenoceptor agonist at dosages ≤ 10 μ g/kg.min in *in vivo* studies on human thermogenesis and lipid utilization.

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IN VIVO
 **β_3 -ADRENERGIC STIMULATION
OF HUMAN THERMOGENESIS
AND LIPID UTILIZATION**

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SLH Schiffelers, EE Blaak, WHM Saris and MA van Baak

Nutrition Toxicology and Environment Research Institute Maastricht,
Department of Human Biology, Maastricht University,
Maastricht, The Netherlands

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Abstract

Aim: To investigate the role of the human β_3 -adrenoceptor in *in vivo* isoprenaline-induced thermogenesis and lipid utilization.

Subjects: In the first study, 8 healthy males with a mean body mass index (BMI) of 22.4 kg/m² (range: 20.0-25.5 kg/m²) and a mean age of 21 y (range: 19-26 y) participated. In the second study, 7 healthy males with a mean BMI of 22.1 kg/m² (range: 20.2-25.5 kg/m²) and a mean age of 23 y (range: 20-27 y) took part.

Design: In the first study, subjects received at random oral dosages of 2.5, 7.5, 15 and 40 mg of nadolol or propranolol (both β_1 - and β_2 -adrenoceptor antagonists), after which isoprenaline (β_1 -, β_2 - and β_3 -adrenoceptor agonist) was infused in an individually determined dosage (range: 19-35 ng/kg.min) which increased energy expenditure by 25% without pretreatment. In the second study, 50, 100 and 200 ng/kg.min of isoprenaline or saline was infused after pretreatment with 80 mg of nadolol.

Measurements: In both studies, energy expenditure and respiratory exchange ratio were measured continuously by indirect calorimetry. At the end of each infusion period, blood samples for the determination of plasma non-esterified fatty acids, glycerol, lactate and potassium concentrations were taken, and tremor score (only first study), heart rate and blood pressure were measured.

Results: In the first study, nadolol or propranolol in dosages $\leq$ 40 mg could not fully block β_1 -adrenoceptor-mediated increases in heart rate and systolic blood pressure and propranolol in dosages $\leq$ 7.5 mg could not fully block the β_2 -adrenoceptor-mediated increase in tremor score during isoprenaline infusion. The found increases in thermogenesis and lipid utilization could therefore be explained by concomitant β_1 - and β_2 -adrenergic stimulation. In the second study, isoprenaline infusion induced a significant rise in heart rate, but no rises in energy expenditure and lipid utilization were found as compared to saline infusion.

Conclusion: No evidence could be found for a β_3 -adrenoceptor-mediated increase in human thermogenesis and lipid utilization during isoprenaline infusion after pretreatment with nadolol or propranolol.

Introduction

The sympathetic nervous system (SNS) plays an important role in energy and substrate metabolism. The effects of the SNS are mediated by α - and β -adrenoceptors. α -Adrenergic stimulation does not affect whole body thermogenesis,^{1,2} whereas non-selective β -adrenergic stimulation significantly increases thermogenesis and lipid utilization.^{2,3} During only β_1 - or only β_2 -adrenergic stimulation, energy expenditure, lipid oxidation and lipolysis increase as well.^{2,4} The role of the β_3 -adrenoceptor in human energy and substrate metabolism is still debated. In rats, β_3 -adrenergic stimulation leads to significant increases in thermogenesis and lipid utilization, which results in weight loss.^{5,6} If this is also the case in humans, specific β_3 -adrenoceptor agonists may be seen as potential anti-obesity drugs, especially since β_3 -adrenergic stimulation causes no tachycardia or tremor. However, the rat β_3 -adrenoceptor differs pharmacologically from the human β_3 -adrenoceptor.^{7,8} Consequently, the specific β_3 -adrenoceptor agonists used in rats are only weak agonists in humans. At this moment, no full β_3 -adrenoceptor agonist is available for administration in humans.

In vitro studies with isolated human fat cells have indicated that the β_1 -, β_2 - and β_3 -adrenoceptor agonist isoprenaline induces β_1 - and β_2 -adrenoceptor-mediated lipolysis in the nanomolar dose range, while β_3 -adrenoceptor-mediated lipolysis is activated at ~ 100-fold higher concentrations.^{9,10} *In vivo* studies with isoprenaline show contradictory results. Isoprenaline infusion leading to nanomolar plasma concentrations predominantly stimulated adipose tissue lipolysis by β_1 -adrenoceptor stimulation, since simultaneous β_1 -adrenoceptor blockade with atenolol completely inhibited the increase in plasma non-esterified fatty acids (NEFA) and glycerol concentrations.¹¹ In another study, isoprenaline-induced lipolysis was completely blocked by the β_1 - and β_2 -adrenoceptor antagonist nadolol.³ However, energy expenditure significantly increased despite β_1 - and β_2 -adrenoceptor blockade, suggesting β_3 -adrenoceptor-mediated thermogenesis.³ The indirectly acting sympathomimetic agent ephedrine significantly increased energy expenditure after pretreatment with a low dose of nadolol, but thermogenesis was completely inhibited after a higher dosage of nadolol.¹² Norepinephrine-induced thermogenesis completely disappeared after intravenous infusion of the β_1 - and β_2 -adrenoceptor antagonist propranolol.² The last two studies therefore provide no evidence for a β_3 -adrenoceptor-mediated effect on thermogenesis in humans.

The discrepancies between these studies may be due to differences in the type and dose of the β -adrenoceptor agonists and antagonists used. In order to investigate whether the human β_3 -adrenoceptor plays a role in isoprenaline-induced human thermogenesis, two studies were performed. In the first study, different dosages of the β_1 - and β_2 -adrenoceptor antagonists nadolol and propranolol were administered to see which minimal dosage is needed to block all β_1 - and β_2 -adrenoceptor-mediated effects of a standardized dose of isoprenaline. Furthermore, we investigated whether a significant increase in thermogenesis and/or lipid utilization would remain after full β_1 - and β_2 -adrenoceptor blockade, which would provide evidence for a functional role of the human β_3 -adrenoceptor. In the second study, isoprenaline was given at a 2- to 10-fold higher infusion rate in combination with nadolol to see whether isoprenaline could induce any β_3 -adrenoceptor-mediated effects on thermogenesis and lipid utilization *in vivo* at these higher concentrations.

Subjects and methods

Subjects

Eight lean, male subjects with a mean body mass index (BMI) of 22.4 kg/m² (range: 20.0-25.5 kg/m²) and a mean age of 21 y (range: 19-26 y) volunteered to participate in the first study. In the second study, seven comparable subjects with a mean BMI of 22.1 kg/m² (range: 20.2-25.5 kg/m²) and a mean age of 23 y (range: 20-27 y) participated. All subjects were in good health and took no medication at the time of the study. The study protocol was approved by the Ethics Committee of Maastricht University and all subjects gave written informed consent before entering the study.

Experimental design

In both studies, subjects came to the laboratory after an overnight fast of at least 10 hours. They came by car or by public transportation in order to minimize the amount of physical activity before the tests. On arrival, a canula was inserted into a forearm vein and subjects rested in semi-supine position during the remainder of the test. All subjects watched television or video during the tests. Room temperature was kept between 23-25 °C and there were at least three days between tests.

During the first visit in the first study, an incremental isoprenaline dose-response test was performed. A 30 min baseline period was followed by increasing doses of 10, 20 and 40 ng/kg.min of isoprenaline (Isoprenaline sulfate, Fresenius, 's Hertogenbosch, The Netherlands), each dose for 30 min. During the test, energy expenditure was continuously measured and heart rate was monitored. When heart rate rose more than 35 beats/min above baseline, isoprenaline infusion was stopped. Using linear regression analysis, the dose of isoprenaline needed for a 25% increase in energy expenditure was calculated for each subject.

During the eight remaining visits in the first study, subjects orally took 2.5, 7.5, 15 or 40 mg of nadolol (Corgard,[®] Bristol-Myers Squibb, Woerden, The Netherlands) or 2.5, 7.5, 15 or 40 mg of propranolol (Inderal,[®] Zeneca, Ridderkerk, The Netherlands), 2 hours before they came to the laboratory. Upon arrival, a 30 min baseline period was followed by a 30 min infusion period in which subjects received their individually determined dosage of isoprenaline. The study design was single blind for subjects and the antagonists were given at random. During the test, energy expenditure and respiratory exchange ratio (RER) were continuously measured and heart rate was monitored. After 20 min of each 30 min period, blood pressure and tremor of the dominant hand were measured and at the end of each 30 min period, a blood sample was taken.

During the second study, subjects ingested 80 mg of nadolol 2 hours before arrival at the laboratory. After a baseline period of 30 min, a continuous infusion of isoprenaline or saline was started. Isoprenaline was infused in increasing dosages of 50, 100 and 200 ng/kg.min, each dosage for 30 min. Saline was administered at the same infusion rates. The study design was single blind for subjects and the order of treatments was randomized. The same parameters were measured as in the first study, except for tremor of the hand.

Clinical methods

Whole body energy expenditure and RER were measured by indirect calorimetry using an open-circuit ventilated hood system.¹³ The volume of air drawn through the hood was measured by a dry-gas meter (Schlumberger, Dordrecht, The Netherlands) and the composition of the inflowing and outflowing air was analyzed by a paramagnetic O₂ analyzer (Servomex, Crowborough, United Kingdom) and an infrared CO₂ analyzer (Hartmann and Braun, Frankfurt, Germany). Airflow rate and the O₂ and CO₂ concentrations of the in- and outgoing air were used to compute O₂ consumption and CO₂ production on-line every 2 min through an automatic acquisition system interfaced with a personal computer. Energy expenditure was calculated according to the formula of Weir.¹⁴ Energy expenditure and RER values were averaged over the last 20 min and 10 min of each infusion step respectively and their means were used for further analysis.

Heart rate was continuously monitored by conventional electrocardiography and mean heart rate was calculated during the last 20 min of each infusion step. Blood pressure was measured after 20 min in each infusion interval by an automatic blood pressure device (Speidel & Keller, Jungingen, Germany) and the mean of five measurements was used for further analysis.

Tremor of the dominant hand was determined with a steadiness test (Steadiness tester 32011, Lafayette Instrument Company, Lafayette, IN, USA) at the end of each infusion period in the first study. Subject's task in this test was to hold a metal-tipped stylus for 15 sec in six progressively smaller hole sizes without touching the sides. If tremor occurred, subjects would touch the sides more often and tremor score consequently increased.

Analytical methods

Blood samples for the determination of plasma glycerol and NEFA concentrations were preserved in sodium-EDTA. Heparinized blood was collected for the determination of plasma lactate and potassium concentrations. Blood samples were immediately centrifuged for 1 min at 7000 x g. Plasma was transferred into microtest tubes, rapidly frozen in liquid nitrogen and stored at -70 °C until further analysis. Plasma NEFA concentration was measured with the NEFA C kit (99475409, WAKO, Neuss, Germany) (coefficient of variation (CV): 2.6%), using a Cobas-Fara centrifugal analyzer (Roche Diagnostica, Basel, Switzerland). Plasma glycerol concentration was measured with a glycerol kit (644200 without triglyceride breakdown step, Boehringer, Mannheim, Germany) (CV: 0.9%) and plasma lactate concentration was measured by the method of Gutmann and Wahlefeld¹⁵ (CV: 2.0%), using a Cobas-Bio centrifugal analyzer (Roche Diagnostica, Basel, Switzerland). Plasma potassium concentration was determined by an ion-selective electrode (Salm & Kipp, Breukelen, The Netherlands) (CV: 0.7%). In each run, standard samples with known concentrations were included for quality control.

Data analysis

Results are presented as mean $\pm$ standard error of the mean (SEM). In the first study, the effect of isoprenaline infusion at the used dosages for each of the used antagonists were assessed by two-way repeated measurements ANOVA. Post hoc testing was done with a paired t-test, corrected according to Bonferroni's inequalities. Differences between the used

antagonists were assessed by factorial ANOVA. In the second study, two-way repeated measurements ANOVA was used to determine significant differences between saline and isoprenaline infusion. Post hoc testing was done with a paired t-test. To assess differences within a trial, one-way repeated measurements ANOVA was used. A P-value < 0.05 was regarded as statistically significant.

Results

First study

The dose of isoprenaline needed for a 25% increase in energy expenditure ranged from 19 to 35 ng/kg.min with a mean value of 27 ng/kg.min.

The used dosages of nadolol had no effect on baseline values of the measured parameters (table 3.1). After 2.5 mg of nadolol, subsequent isoprenaline infusion significantly increased energy expenditure (figure 3.1), plasma NEFA and glycerol concentrations (figure 3.2), heart rate and systolic blood pressure (figure 3.3) and significantly lowered RER (figure 3.1) and diastolic blood pressure (figure 3.3). Plasma lactate and potassium concentrations and tremor score were not significantly affected (table 3.2). After pretreatment with 7.5, 15 and 40 mg of nadolol, isoprenaline infusion resulted only in significant increases in heart rate and systolic blood pressure and a significant decrease in diastolic blood pressure (7.5 mg only) and RER (40 mg only). All other parameters remained unchanged.

The used dosages of propranolol did not affect baseline parameters (table 3.1). Pretreatment with 2.5, 7.5 and 15 mg of propranolol led to significant increases in plasma NEFA and glycerol concentrations (figure 3.2), heart rate and systolic blood pressure (figure 3.3) and significant decreases in RER (figure 3.1) and diastolic blood pressure (figure 3.3). Energy expenditure (figure 3.1) and tremor score (table 3.2) only significantly increased during isoprenaline infusion after pretreatment with 2.5 and 7.5 mg of propranolol, but not after 15 and 40 mg of propranolol. Plasma lactate and potassium concentrations were not

Figure 3.1 Changes in energy expenditure and respiratory exchange ratio during isoprenaline infusion after pretreatment with 2.5, 7.5, 15 or 40 mg of nadolol or propranolol. Values are mean $\pm$ SEM for 8 subjects. Paired t-test: baseline vs isoprenaline: * P<0.05, ** P<0.01, *** P<0.001.

affected by propranolol (table 3.1). After administration of 40 mg of propranolol, isoprenaline infusion still led to significant increases in heart rate and systolic blood pressure and a significant decrease in tremor score.

In summary, both nadolol and propranolol antagonized the changes induced by isoprenaline. At the highest dosages of nadolol and propranolol still significant changes in heart rate and systolic blood pressure were found during isoprenaline infusion; most other parameters no longer differed significantly from baseline. At the same dose of antagonist, the antagonizing effect of nadolol on changes in RER, plasma NEFA concentration, tremor score, systolic blood pressure (factorial ANOVA for antagonist: all $P < 0.01$) and heart rate (factorial ANOVA for antagonist: $P < 0.001$) was more pronounced than the antagonizing effect of propranolol.

Second study

Infusion of saline or increasing dosages of isoprenaline significantly increased energy expenditure, plasma NEFA, glycerol, lactate and potassium concentrations, heart rate and systolic blood pressure after pretreatment with 80 mg of nadolol. RER significantly decreased and diastolic blood pressure remained unchanged during saline or isoprenaline infusion (figure 3.4, table 3.3).

Only the increase in heart rate was significantly higher during isoprenaline infusion as compared to saline infusion (two-way repeated measurements ANOVA for heart rate $\times$ treatment: $P < 0.001$) (figure 3.4). All other parameters did not differ between treatments.

Discussion

The first study was performed to investigate whether any significant increases in isoprenaline-induced thermogenesis, lipid oxidation and/or lipolysis would remain after full β_1 - and β_2 -adrenoceptor blockade, which would provide evidence for a functional role of the

Figure 3.2 Changes in plasma non-esterified fatty acids (NEFA) and glycerol concentrations during isoprenaline infusion after pretreatment with 2.5, 7.5, 15 or 40 mg of nadolol or propranolol. Values are mean $\pm$ SEM for 8 subjects. Paired t-test: baseline vs isoprenaline: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 3.1 Baseline values of the measured parameters after pretreatment with nadolol or propranolol.

Parameter	Nadolol (mg)				Propranolol (mg)			
	2.5	7.5	15	40	2.5	7.5	15	40
EE (kJ/min)	5.71 ± 0.17	5.74 ± 0.18	5.85 ± 0.17	5.76 ± 0.22	5.85 ± 0.21	5.89 ± 0.24	5.97 ± 0.24	5.60 ± 0.19
RER	0.87 ± 0.01	0.86 ± 0.02	0.86 ± 0.02	0.87 ± 0.02	0.84 ± 0.02	0.86 ± 0.02	0.86 ± 0.02	0.82 ± 0.01
NEFA (μmol/l)	279 ± 45	335 ± 52	278 ± 32	272 ± 32	358 ± 113	349 ± 87	264 ± 32	409 ± 46
Glycerol (μmol/l)	49.9 ± 3.8	51.9 ± 3.8	50.4 ± 3.6	53.6 ± 4.0	46.2 ± 4.6	51.9 ± 3.5	47.9 ± 3.3	58.2 ± 3.0
Lactate (mmol/l)	1.35 ± 0.06	1.25 ± 0.11	1.45 ± 0.13	1.44 ± 0.12	1.52 ± 0.11	1.37 ± 0.08	1.43 ± 0.12	1.17 ± 0.09
Potassium (mmol/l)	4.05 ± 0.09	4.05 ± 0.07	4.00 ± 0.06	4.08 ± 0.09	3.97 ± 0.10	4.13 ± 0.06	4.11 ± 0.08	3.99 ± 0.08
Tremor score	6.19 ± 0.92	6.77 ± 1.72	5.52 ± 1.02	4.63 ± 0.70	6.94 ± 1.47	6.06 ± 0.82	6.77 ± 1.31	7.75 ± 1.52
Heart rate (beats/min)	50 ± 2	49 ± 3	47 ± 2	47 ± 3	52 ± 3	53 ± 3	52 ± 2	49 ± 3
Systolic BP (mmHg)	114 ± 3	109 ± 2	113 ± 5	113 ± 3	119 ± 4	119 ± 4	116 ± 3	113 ± 3
Diastolic BP (mmHg)	75 ± 3	73 ± 2	78 ± 3	74 ± 2	73 ± 3	74 ± 2	72 ± 2	77 ± 2

Values are mean ± SEM for 8 subjects. EE: energy expenditure; RER: respiratory exchange ratio; NEFA: non-esterified fatty acids; BP: blood pressure.

Table 3.2 Changes in plasma lactate and potassium concentrations and tremor score compared to baseline during isoprenaline infusion after pretreatment with nadolol or propranolol.

Parameter	Nadolol (mg)				Propranolol (mg)			
	2.5	7.5	15	40	2.5	7.5	15	40
Δ Lactate (mmol/l)	0.04 ± 0.06	0.01 ± 0.05	0.05 ± 0.05	-0.08 ± 0.05	0.24 ± 0.20	0.01 ± 0.08	-0.01 ± 0.14	-0.01 ± 0.04
Δ Potassium (mmol/l)	-0.03 ± 0.06	0.07 ± 0.05	0.03 ± 0.02	0.04 ± 0.03	-0.08 ± 0.10	-0.06 ± 0.05	-0.08 ± 0.06	0.10 ± 0.03
Δ Tremor score	2.38 ± 1.07	1.38 ± 0.87	0.21 ± 0.83	0.92 ± 0.86	5.31 ± 1.36**	3.31 ± 1.14*	3.26 ± 1.55	-1.24 ± 0.62*

Values are mean ± SEM for 8 subjects. Paired t-test: baseline vs isoprenaline: *P<0.05, **P<0.01.

Figure 3.3 Changes in heart rate, and systolic and diastolic blood pressure during isoprenaline infusion after pretreatment with 2.5, 7.5, 15 or 40 mg of nadolol or propranolol. Values are mean $\pm$ SEM for 8 subjects. Paired t-test: baseline vs isoprenaline: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

human β_3 -adrenoceptor during isoprenaline infusion. To assure that all β_1 -adrenoceptor-mediated effects of isoprenaline were blocked by nadolol or propranolol, heart rate and systolic blood pressure were measured as indicators for β_1 -adrenergic stimulation.¹⁶ Administration of nadolol or propranolol in a range of 2.5 to 40 mg could not prevent a significant isoprenaline-induced increase in heart rate and systolic blood pressure. This suggests that no complete β_1 -adrenoceptor blockade was achieved with the dosages of antagonists used. To control for β_2 -adrenergic stimulation, plasma lactate and potassium concentrations and tremor score were measured as specific indicators for β_2 -adrenergic stimulation.¹⁶ An isoprenaline-induced increase in tremor score only occurred after pretreatment with 2.5 and 7.5 mg of propranolol. Otherwise, no indications for β_2 -adrenergic stimulation could be found, indicating that after pretreatment with 2.5 to 40 mg of nadolol and 15 and 40 mg of propranolol all β_2 -adrenoceptor-mediated effects of isoprenaline were blocked. Overall however, no complete β_1 - and β_2 -adrenoceptor blockade was achieved after pretreatment with nadolol nor with propranolol at dosages ≤ 40 mg. This suggests that the found increases in thermogenesis, lipid oxidation, as indicated by a decrease in RER, and lipolysis, as indicated by increases in plasma NEFA and glycerol concentrations, in the first study might be explained by β_1 - and/or β_2 -adrenergic stimulation and no evidence can be provided for a functional role of the β_3 -adrenoceptor in human thermogenesis and lipid utilization during the infusion of ~ 30 ng/kg.min isoprenaline.

In the second study, isoprenaline infusion rates were increased to 50, 100 and 200 ng/kg.min. The nadolol dosage was increased to 80 mg, since the 40 mg of nadolol used in

Table 3.3 Changes in measured parameters compared to baseline during saline or isoprenaline infusion after pretreatment with 80 mg of nadolol.

Parameter	Treatment	Isoprenaline (ng/kg.min)			ANOVA	
		50	100	200	Parameter	Par x Treatm
Δ Respiratory exchange ratio	Saline	-0.007 $\pm$ 0.010	-0.015 $\pm$ 0.007	-0.020 $\pm$ 0.006	P<0.01	NS
	Isoprenaline	-0.022 $\pm$ 0.005	-0.040 $\pm$ 0.010	-0.031 $\pm$ 0.016		
Δ NEFA (μ mol/l)	Saline	21 $\pm$ 33	93 $\pm$ 54	100 $\pm$ 65	P<0.001	NS
	Isoprenaline	31 $\pm$ 34	98 $\pm$ 42	178 $\pm$ 58		
Δ Glycerol (μ mol/l)	Saline	3.2 $\pm$ 2.7	11.3 $\pm$ 5.0	18.9 $\pm$ 7.0	P<0.01	NS
	Isoprenaline	-6.0 $\pm$ 4.2	0.9 $\pm$ 4.2	5.0 $\pm$ 5.9		
Δ Lactate (mmol/l)	Saline	0.05 $\pm$ 0.03	0.07 $\pm$ 0.04	0.06 $\pm$ 0.04	P<0.05	NS
	Isoprenaline	0.01 $\pm$ 0.02	0.04 $\pm$ 0.02	0.04 $\pm$ 0.03		
Δ Potassium (mmol/l)	Saline	0.23 $\pm$ 0.08	0.24 $\pm$ 0.13	0.27 $\pm$ 0.10	P<0.05	NS
	Isoprenaline	0.22 $\pm$ 0.12	0.28 $\pm$ 0.13	-0.08 $\pm$ 0.17		
Δ Diastolic BP (mmHg)	Saline	1 $\pm$ 1	1 $\pm$ 3	2 $\pm$ 3	NS	NS
	Isoprenaline	-1 $\pm$ 2	1 $\pm$ 3	3 $\pm$ 5		

Values are mean $\pm$ SEM for 7 subjects. NEFA: non-esterified fatty acids; BP: blood pressure.

Figure 3.4 Changes in energy expenditure, heart rate and systolic blood pressure during saline (●) or isoprenaline (Δ) infusion after pretreatment with 80 mg of nadolol. Values are mean $\pm$ SEM for 7 subjects. One-way repeated measurements ANOVA: [#] $P < 0.05$, ^{***} $P < 0.001$. Paired t-test: saline vs isoprenaline: ^{**} $P < 0.01$, ^{***} $P < 0.001$.

the first study did not completely block all β_1 -adrenoceptor-mediated effects on heart rate and systolic blood pressure. Nadolol was chosen, since its blocking properties were more pronounced as compared to those of propranolol, as shown in the first study, and higher dosages of propranolol might partly block β_3 -adrenoceptors, as shown by Emorine *et al.*¹⁷ In the second study, isoprenaline infusion did not cause any significant changes in energy expenditure, lipid oxidation and lipolysis as compared to saline infusion. Only a significant increase in heart rate occurred as compared to saline which might indicate that non-selective β -adrenergic stimulation by a high dosage of isoprenaline overcomes β_1 -adrenoceptor blockade. Thus, we conclude that after pretreatment with 80 mg of nadolol, isoprenaline infusion at a rate ≤ 200 ng/kg.min does not provide evidence for a β_3 -adrenoceptor-mediated increase in thermogenesis and lipid utilization *in vivo* in humans. *In vitro* studies in CHO-cells show that 3.9 nmol/l isoprenaline is required to produce a half-maximal response for the human β_3 -adrenoceptor.¹⁷ With an infusion rate of 200 ng/kg.min, plasma levels of ~ 10 nmol/l are achieved, suggesting that the isoprenaline concentration might have been high enough to find an effect of β_3 -adrenoceptor stimulation.

Wheeldon *et al.*³ performed a similar study. They infused isoprenaline at a rate (0.5-3.0 $\mu\text{g/min}$) which increased the individual heart rate by 60 beats/min and resulted in a 30% increase in energy expenditure. Pretreatment with 20 and 80 mg of nadolol completely blocked the β_1 -adrenoceptor-specific change in heart rate and the β_2 -adrenoceptor-specific change in finger tremor. Concomitant isoprenaline infusion induced a significant 13% and 10% increase in thermogenesis after pretreatment with 20 and 80 mg of nadolol respectively, which was considered to be due to β_3 -adrenergic stimulation. The isoprenaline-induced

increases in lipid oxidation and lipolysis were completely blocked by 20 and 80 mg of nadolol, suggesting that fat metabolism was probably not stimulated via β_3 -adrenoceptors. According to our study, β_1 -adrenoceptors are not fully blocked at the nadolol concentrations used and the found increase in thermogenesis might therefore be explained by concomitant β_1 -adrenergic stimulation.

Liu *et al.*¹² performed a study in which ephedrine was given to stimulate the release of norepinephrine from sympathetic nerve endings, leading to high local norepinephrine levels. 30 mg of ephedrine produced a 6.6% increase in energy expenditure. After pretreatment with 2.5 mg of nadolol, energy expenditure still significantly increased by 2.3% after ephedrine administration. After pretreatment with 5 and 10 mg of nadolol, no changes in thermogenesis were found. All ephedrine-induced changes in heart rate and blood pressure were blocked after pretreatment with 2.5 mg of nadolol according to authors, but they neglected to correct for baseline values which were also affected by nadolol. According to our recalculations, ephedrine induced an increase in systolic blood pressure after pretreatment with 2.5 mg of nadolol and an increase in both systolic blood pressure and heart rate with 5 mg of nadolol. This suggests that β_1 -adrenoceptors were not completely blocked with these lower dosages of nadolol, which is in accordance with our findings and those of Wheeldon *et al.*³ The observed significant increase in thermogenesis by Liu *et al.* might therefore be due to concomitant β_1 -adrenergic stimulation and not to β_3 -adrenergic stimulation.

Finally, Blaak *et al.*² showed that infusion of the α -, β_1 -, β_2 - and β_3 -adrenoceptor agonists epinephrine (15-60 ng/kg.min) or norepinephrine (25-100 ng/kg.min) in combination with propranolol (bolus: 195 μ g/kg, infusion: 0.6 μ g/kg.min) did not change energy expenditure, but significantly increased mean arterial blood pressure by α -adrenoceptor stimulation and consequently, significantly decreased heart rate. Blaak *et al.* concluded that the used dosages of epinephrine and norepinephrine could not induce a β_3 -adrenoceptor-mediated increase in thermogenesis.

Above mentioned studies are the only ones published until now that have selectively tried to stimulate β_3 -adrenoceptors. Other studies used relatively unselective β_3 -adrenoceptor agonists to determine the effect of β_3 -adrenergic stimulation on human thermogenesis and lipid utilization *in vivo*. The thermogenic compound Ro 16-8714 not only increased energy expenditure, but also had significant effects on heart rate and systolic blood pressure, indicating that part of its effect was mediated via β_1 -adrenoceptors.¹⁸ Clinical studies with BRL 26830A showed that this β_3 -adrenoceptor agonist not only induced thermogenesis, but also tremor, suggesting additional β_2 -adrenergic stimulation.¹⁹ The β_3 -adrenoceptor agonist BRL 35135 significantly increased thermogenesis and lipid utilization, but also induced a significant decrease in serum potassium concentration, indicating additional β_2 -adrenergic stimulation. After pretreatment with 20 mg of nadolol, BRL 35135 still caused a small, but significant increase in thermogenesis, but lipid oxidation and lipolysis were unaffected.²⁰ Heart rate and blood pressure were not measured in this study, so it is unknown whether concomitant β_1 -adrenergic stimulation could explain this effect.

Although no clear evidence is available from *in vivo* studies, we still feel that the β_3 -adrenoceptor may still play a role in human thermogenesis and lipid utilization. After the administration of CGP 12177 (selective β_3 -adrenoceptor agonist with β_1 - and β_2 -adrenoceptor antagonist properties), significant increases in glycerol release were found both *in vitro*^{21,22}

from omental and abdominal fat cells and *in situ*^{23,24} from abdominal subcutaneous adipose tissue by using the microdialysis technique. The increase in glycerol concentration was only 30% of that found after isoprenaline administration,^{21,22,24} but was not affected by the addition of propranolol,^{22,23} indicating that CGP 12177 is a weak, but selective β_3 -adrenoceptor agonist. No studies are available on *in vivo* effects of CGP 12177. Furthermore, several studies have shown that an increase in plasma NEFA concentration by infusing a lipid or lipid heparin mixture induces increases in lipid oxidation²⁵ and thermogenesis.^{26,27} Thus, if CGP 12177 or a full β_3 -adrenoceptor agonist is capable of increasing lipolysis to the extent that plasma NEFA concentrations increase, significant effects on lipid oxidation and thermogenesis may be expected.

In conclusion, the data from this study indicate that the used dosages of nadolol and propranolol did not fully block all β_1 - and β_2 -adrenoceptor-mediated effects at the given concentrations isoprenaline. Furthermore, no evidence could be provided for a β_3 -adrenoceptor-mediated increase in human energy expenditure, lipid oxidation and lipolysis during isoprenaline infusion at dosages ≤ 200 ng/kg.min. However, with the development of more selective β_3 -adrenoceptor agonists in the future, a possible role for the β_3 -adrenoceptor in *in vivo* human thermogenesis and lipid utilization might still be demonstrated.

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INHIBITION OF LIPOLYSIS REDUCES β_1 -ADRENOCEPTOR-MEDIATED THERMOGENESIS IN MEN

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SLH Schiffelers, EMC Brouwer, WHM Saris and MA van Baak

Nutrition Toxicology and Environment Research Institute Maastricht,
Department of Human Biology, Maastricht University,
Maastricht, The Netherlands

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Abstract

Aim: To investigate whether the increase in energy expenditure and lipid oxidation during β_1 -adrenergic stimulation is caused by the concomitant increase in lipolysis.

Subjects: Twelve healthy male volunteers with a mean body mass index of 22.8 kg/m² (range: 18.5-27.8 kg/m²) and a mean age of 25 y (range: 19-41 y).

Design: Subjects participated in three trials: trial *no-LIP*:- inhibition of lipolysis by pretreatment with acipimox (250 mg) followed by saline infusion; trial *-/BETA*: no pretreatment, dobutamine infusion (5 µg/kg.min) to stimulate β_1 -adrenoceptors; trial *no-LIP/BETA*: pretreatment with acipimox followed by dobutamine infusion.

Measurements: Energy expenditure, respiratory exchange ratio and lipid and carbohydrate oxidation were continuously measured by indirect calorimetry. At the end of each infusion period, a blood sample for the determination of plasma non-esterified fatty acids (NEFA), glycerol, glucose, insulin, lactate and potassium concentrations were taken, and heart rate and blood pressure were measured.

Results: Inhibition of lipolysis lowered baseline plasma glycerol and NEFA concentrations, did not affect baseline energy expenditure, and decreased lipid oxidation and increased carbohydrate oxidation (*no-LIP*:-). Glycerol and NEFA concentrations increased significantly during β_1 -adrenergic stimulation alone (*-/BETA*). Concomitant administration of acipimox prevented a substantial part of the increase in lipolysis during β_1 -adrenergic stimulation, but increases in plasma glycerol and NEFA levels remained significant (*no-LIP/BETA*). Energy expenditure and lipid oxidation increased significantly during β_1 -adrenergic stimulation (*-/BETA*), but this increase was significantly lower when lipolysis was inhibited (*no-LIP/BETA*).

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 men.

Introduction

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, while α_1 - and α_2 -adrenoceptors do not play important roles.^{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 a.o. 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 increases in adipose tissue lipolysis^{5,6} and in whole body^{7,8} and skeletal muscle⁵ lipid oxidation. Infusion of the non-selective β -agonist isoprenaline also leads to increases in lipolysis and lipid oxidation.⁹

Recent studies showed an increase in lipolysis, lipid oxidation and thermogenesis 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 is therefore not likely to increase energy expenditure and lipid oxidation by direct stimulation of the skeletal muscle. We therefore hypothesize that the elevated levels of non-esterified fatty acids (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 see 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 volunteers with a mean age of 25 y (range: 19-41 y) participated in this study. Their average body weight was 73.8 kg (range: 57.2-93.1 kg), their average body mass index was 22.8 kg/m² (range: 18.5-27.8 kg/m²) and their average fat percentage was 12.3% (range: 5.2-22.4%). The subjects were healthy and took no medication at the time of the study. The study was reviewed and approved by the Ethics Committee of Maastricht University and all subjects gave informed consent before participating in the study.

Experimental design

Subjects participated in three trials, which were performed in random order: in trial *no-LIP*-, lipolysis was inhibited by pretreatment with acipimox (Nedios,[®] Byk, Zwanenburg, The Netherlands) after which saline was given as a placebo infusion; in trial *-BETA*-, no pre-

treatment was given, only dobutamine (Dobax,[®] Byk, Zwanenburg, The Netherlands) was infused to stimulate β_1 -adrenoceptors; in trial *no*-LIP/BETA, pretreatment with acipimox was followed by an infusion with dobutamine. No placebo capsule was given for acipimox, since blinding could not be obtained. The flushing after acipimox administration would clearly distinguish acipimox from placebo. The study design was single-blind for dobutamine. Subjects were fasted overnight and came to the laboratory by car or by bus to minimize the amount of physical activity before the tests. Experiments started at 8:00 AM or 9:30 AM, each subject always starting at the same time. At least 3 days separated consecutive tests. Room temperature was kept at 21–23 °C.

The capsule of acipimox (250 mg) was taken 90 min before the start of the experiment (figure 4.1). On arrival at the laboratory, a canula was inserted into a forearm vein after which measurements of energy expenditure, respiratory exchange ratio (RER), heart rate and blood pressure were started with the subject in semi-supine position. After a 30 min baseline period, a continuous infusion of dobutamine (5 $\mu\text{g}/\text{kg}\cdot\text{min}$) or saline was given for 30 min. At the end of each 30 min period, a venous blood sample was taken. The infusion was stopped prematurely if heart rate had increased by more than 30 beats/min and/or mean blood pressure had risen by more than 30 mmHg. This occurred once during the study.

Figure 4.1 Experimental study design. Twelve male subjects participated in three randomized trials: *no*-LIP/-: inhibition of lipolysis by pretreatment with acipimox (250 mg) followed by a saline infusion, -/BETA: no pretreatment, dobutamine infusion (5 $\mu\text{g}/\text{kg}\cdot\text{min}$) to stimulate β_1 -adrenoceptors and *no*-LIP/BETA: pretreatment with acipimox followed by dobutamine infusion.

Clinical methods

Body density was determined by hydrostatic weighing with simultaneous lung volume measurement (Volugraph 2000, Mijnhardt, Bunnik, The Netherlands). Body composition was calculated according to the formula of Siri.¹³

Whole body energy expenditure and RER were measured by an 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 the in- 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 the O_2 and CO_2 concentrations 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. 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 min of each 30 min period during which 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 the energy expended, and averaged over the last 10 min of each 30 min period.

Heart rate was monitored continuously by conventional electrocardiography and was recorded at the end of each 5 min interval. Heart rate values were averaged over the last 10 min of each 30 min period and used for further analysis. Blood pressure was measured by an automated blood pressure device (Tonoprint, Speidel & Keller, Jungingen, Germany) after 20 min in each 30 min interval. The means of four measurements per interval were computed and used for further analysis.

Analytical methods

Blood samples were preserved in sodium-EDTA (20 μ l 7.5% w/v Na-EDTA per ml blood) and immediately centrifuged for 10 min at 800 $\times g$ at 4 °C. Plasma was transferred into microtest tubes, rapidly frozen in liquid nitrogen and stored at -60 °C until further analysis. Plasma NEFA concentration was measured with the NEFA C kit (99475409, WAKO, Neuss, Germany) and plasma glycerol concentration was measured with a glycerol kit (148270, Boehringer, Mannheim, Germany), both on a Cobas-Fara centrifugal analyzer (Roche Diagnostica, Basel, Switzerland). Plasma glucose concentration was measured with a glucose kit (Unimate 5, 0736724, Roche Diagnostica, Basel, Switzerland) and plasma lactate concentration was measured by the method of Gutmann and Wahlefeld,¹⁶ both on a Cobas-Bio centrifugal analyzer (Roche Diagnostica, Basel, Switzerland). Plasma insulin concentration was determined with a double-antibody radioimmunoassay (Insulin RIA 100, Pharmacia, Uppsala, Sweden).

Data analysis

All data are presented as mean $\pm$ standard error of the mean (SEM). Comparison of data between the baseline and the infusion period within a trial was done with a paired t-test. Comparison of the data at baseline or during the infusion period among trials was done

Figure 4.2 Energy expenditure and respiratory exchange ratio 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). Values are mean $\pm$ SEM for 12 subjects. Paired t-test corrected for Bonferroni's inequalities: baseline vs infusion period: * $P < 0.05$, *** $P < 0.001$.

with a two-way repeated measurements ANOVA. Post hoc testing was done with a paired t-test and the P-values of the post hoc comparisons were corrected according to Bonferroni's inequalities. A P-value < 0.05 was regarded as statistically significant.

Results

Energy expenditure, RER and substrate oxidation

Inhibition of lipolysis did not affect energy expenditure at baseline (figure 4.2). Energy expenditure increased from 5.15 ± 0.16 to 6.11 ± 0.26 kJ/min ($P < 0.001$) during β_1 -adrenergic stimulation alone and from 5.28 ± 0.17 to 5.71 ± 0.19 kJ/min ($P < 0.01$) during β_1 -adrenergic stimulation with concomitant inhibition of lipolysis. The increase in energy expenditure was significantly reduced when lipolysis was inhibited (-/BETA vs no-LIP/BETA: 0.93 ± 0.15 vs 0.43 ± 0.10 kJ/min, $P < 0.05$). RER increased significantly at baseline when lipolysis was inhibited (figure 4.2). 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 (figure 4.3). 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 significantly increased from 59 ± 4 to 73 ± 6 mg/min ($P < 0.01$) during β_1 -adrenergic stimulation alone. When lipolysis was inhibited, lipid oxidation increased from 34 ± 5 to 39 ± 6 mg/min during β_1 -adrenergic stimulation ($P < 0.05$). The increase in lipid oxidation was significantly reduced when lipolysis was inhibited (-/BETA vs no-LIP/BETA: 15 ± 4 vs 6 ± 2 mg/min, $P < 0.05$).

Figure 4.3 Lipid and carbohydrate oxidation 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). Values are mean $\pm$ SEM for 12 subjects. Paired t-test corrected for Bonferroni's inequalities: baseline vs infusion period: * $P < 0.05$, ** $P < 0.01$.

Blood parameters

Baseline plasma concentrations of glycerol and NEFA decreased significantly after inhibition of lipolysis (figure 4.4). Plasma glycerol concentration did not change during saline infusion, but plasma NEFA concentration decreased slightly further from 113 to 93 $\mu\text{mol/l}$ ($P < 0.001$). Plasma glycerol and NEFA concentrations increased significantly during β_1 -adrenergic stimulation alone (baseline vs infusion period, glycerol: 65.0 ± 5.3 vs 117.0 ± 10.9 $\mu\text{mol/l}$, NEFA: 362 ± 24 vs 954 ± 89 $\mu\text{mol/l}$, both $P < 0.001$). After inhibition of lipolysis, glycerol and NEFA concentrations still increased slightly, but significantly, during β_1 -adrenergic stimulation (baseline vs infusion period, glycerol: 40.4 ± 2.2 vs 44.8 ± 2.2 $\mu\text{mol/l}$, NEFA: 118 ± 17 vs 160 ± 19 $\mu\text{mol/l}$, both $P < 0.05$). The increases in plasma glycerol and NEFA concentrations were significantly reduced when lipolysis was inhibited ($-/\text{BETA}$ vs $\text{no-LIP}/\text{BETA}$, Δ glycerol: 52.0 ± 10.2 vs 4.4 ± 1.8 $\mu\text{mol/l}$, Δ NEFA: 591 ± 87 vs 42 ± 16 $\mu\text{mol/l}$, both $P < 0.001$).

Figure 4.4 Plasma non-esterified fatty acids (NEFA) and glycerol concentrations 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). Values are mean $\pm$ SEM for 12 subjects. Paired t-test corrected for Bonferroni's inequalities: baseline vs infusion period: * $P < 0.05$, *** $P < 0.001$.

Plasma glucose concentration decreased at baseline after inhibition of lipolysis (table 4.1). Plasma glucose levels decreased significantly during β_1 -adrenergic stimulation. This decrease was not affected by inhibition of lipolysis ($-/\text{BETA}$ vs $\text{no-LIP}/\text{BETA}$: 0.33 ± 0.04 vs 0.33 ± 0.03 $\mu\text{mol/l}$, NS). Baseline plasma insulin concentration was not affected by inhibition of lipolysis (table 4.1). Plasma insulin levels increased significantly during β_1 -adrenergic stimulation alone ($-/\text{BETA}$), but did not change when lipolysis was inhibited ($\text{no-LIP}/-$ and $\text{no-LIP}/\text{BETA}$). Plasma lactate concentrations were similar at baseline (table 4.1). Plasma lactate concentrations decreased significantly during β_1 -adrenergic stimulation without preceding intervention ($-/\text{BETA}$). Plasma potassium levels were significantly different among trials at baseline and during infusion (table 4.1). Post hoc testing showed no statistically significant differences between pairs of groups at baseline. Plasma potassium concentration increased significantly after the infusion of saline ($\text{no-LIP}/-$), but remained similar during β_1 -adrenergic stimulation ($-/\text{BETA}$ and $\text{no-LIP}/\text{BETA}$).

Table 4.1 Plasma concentrations of metabolites during baseline with or without inhibition of lipolysis (*no*-LIP) by acipimox and during infusion of saline or dobutamine to stimulate β_1 -adrenoceptors (BETA).

	<i>no</i> -LIP/-		-/BETA		<i>no</i> -LIP/BETA		ANOVA	
	Baseline	Infusion	Baseline	Infusion	Baseline	Infusion	Baseline	Infusion
Glucose (mmol/l)	4.72 ± 0.10	4.57 ± 0.12	5.06 ± 0.09 ^a	4.73 ± 0.07 ^c	4.88 ± 0.13	4.56 ± 0.13 ^c	P<0.001	NS
Insulin (mU/l)	5.33 ± 0.48	4.97 ± 0.50	5.94 ± 0.67	7.66 ± 0.67 ^{bd}	5.44 ± 0.48	5.78 ± 0.54 ^f	NS	P<0.01
Lactate (μmol/l)	771 ± 56	748 ± 51	751 ± 55	666 ± 49 ^b	782 ± 41	772 ± 40	NS	NS
Potassium (mmol/l)	4.16 ± 0.08	4.26 ± 0.09 ^a	4.14 ± 0.06	4.17 ± 0.07	4.32 ± 0.08	4.35 ± 0.08 ^{gh}	P<0.05	P<0.05

Values are mean ± SEM for 12 subjects. Paired t-test corrected for Bonferroni inequalities: within trials: baseline vs infusion period: ^a P<0.05, ^b P<0.01, ^c P<0.001; between trials: -/BETA vs *no*-LIP/-: ^d P<0.05, ^e P<0.01, *no*-LIP/BETA vs -/BETA: ^f P<0.05, ^g P<0.01, *no*-LIP/BETA vs *no*-LIP/-: ^h P<0.05.

Heart rate and blood pressure

Heart rate did not differ among trials at baseline, nor did it change during saline infusion or during β_1 -adrenergic stimulation (baseline vs infusion period, *no*-LIP/-: 58 ± 3 vs 58 ± 3 ; -/BETA: 56 ± 3 vs 57 ± 2 ; *no*-LIP/BETA: 55 ± 3 vs 56 ± 2 beats/min). Baseline values for systolic and diastolic blood pressure were not different among trials (*no*-LIP/-: $119/80 \pm 2/2$; -/BETA: $122/76 \pm 2/2$; *no*-LIP/BETA: $120/80 \pm 2/2$ mmHg). Saline infusion had no effect on systolic and diastolic blood pressure. Systolic blood pressure increased ($P < 0.001$) and diastolic blood pressure decreased ($P < 0.05$) to a similar extent during β_1 -adrenergic stimulation alone and during β_1 -adrenergic stimulation with concomitant inhibition of lipolysis (-/BETA: $171/65 \pm 4/3$; *no*-LIP/BETA: $167/69 \pm 4/3$ mmHg) (figure 4.5).

Figure 4.5 Changes in systolic (■) and diastolic (□) blood pressure between baseline (with or without inhibition of lipolysis (*no*-LIP) by acipimox) and infusion period (saline or dobutamine to stimulate β_1 -adrenoceptors (BETA)). Values are mean $\pm$ SEM for 12 subjects. Paired t-test corrected for Bonferroni's inequalities: baseline vs infusion period: ** $P < 0.01$, *** $P < 0.001$.

Almost every subject increased his energy expenditure, lipid oxidation and lipolysis rates during β_1 -adrenergic stimulation with or without concomitant inhibition of lipolysis. Only two of the twelve subjects showed no change in their lipid oxidation lipolysis rates during the *no*-LIP/BETA trial. No subject responded in an opposite direction. Expressing above mentioned data per kg fat free mass did not change the interpretation of this study.

Discussion

The present study was performed to investigate the mechanism behind 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 see 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 cAMP 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 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 the full 60 min 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 levels rose by 80% and plasma NEFA levels by 163% (-/BETA). This is in accordance with earlier studies by Green *et al.*¹¹ and Bhatt *et al.*¹⁰ Inhibition of lipolysis with acipimox for the greater part prevented the increases in plasma glycerol and NEFA concentrations during β_1 -adrenergic stimulation, but plasma glycerol levels still rose by 11% and plasma NEFA levels by 36% (*no*-LIP/BETA). This indicates that lipolysis was not completely blocked by acipimox. Energy expenditure increased by 19% during β_1 -adrenergic stimulation alone. This is also in accordance with the study by Green *et al.*,¹¹ who found an increase of 17%. Inhibition of lipolysis reduced the increase in energy expenditure during β_1 -adrenergic stimulation to 8%. Lipid oxidation increased by 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 by 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 had occurred. This is in agreement with other studies that have indicated 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 ($\geq 6 \mu\text{g/kg}\cdot\text{min}$).¹⁸ In a study by Green *et al.*¹¹ (using the same dose of dobutamine as in our study), no change in plasma epinephrine concentration and a decrease in plasma norepinephrine concentration 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 authors^{8,19} that the catecholamine-induced increase in whole body energy expenditure may partly be 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 with 19% during β_1 -adrenergic stimulation alone and with 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 increases 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 to -/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 directly be increased by dobutamine. Whether an increased plasma NEFA concentration without β_1 -adrenergic stimulation can increase energy expenditure is still debated. After infusing a lipid heparin mixture which increases plasma NEFA concentration, some studies find no changes in energy expenditure²¹ or lipid oxidation,²² whereas others^{23,24} find significant increases in energy expenditure. Our study is not able to answer the question 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 oxidizing more lipids. 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 increases 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.

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β_1 - AND β_2 -ADRENOCEPTOR-MEDIATED THERMOGENESIS AND LIPID UTILIZATION IN OBESE AND LEAN MEN

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SLH Schiffelers,¹ WHM Saris,¹ F Boomsma² and MA van Baak¹

¹Nutrition Toxicology and Environment Research Institute Maastricht,
Department of Human Biology, Maastricht University, Maastricht,

²Department of Internal Medicine I, University Hospital Dijkzigt,
Erasmus University, Rotterdam,
The Netherlands.

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Abstract

Aim: To elucidate the roles of the β_1 - and the β_2 -adrenoceptor in thermogenesis and lipid utilization in obese and lean men.

Subjects: The β_1 -adrenoceptor study was performed in 9 obese and 10 lean men with a mean body mass index (BMI) of 32.4 kg/m² (range: 29.1-36.0 kg/m²) and 23.7 kg/m² (range: 20.4-26.6 kg/m²) and a mean age of 46 y (range: 39-51 y) and 41 y (range: 34-50 y), respectively. The β_2 -adrenoceptor study was performed in 10 obese and 11 lean men with a mean BMI of 33.2 kg/m² (range: 29.5-37.1 kg/m²) and 23.4 kg/m² (range: 19.1-26.4 kg/m²) and a mean age of 47 y (range: 40-50 y) and 43 y (range: 34-50 y), respectively.

Design: The β_1 -adrenoceptor study consisted of four study periods during which subjects received consecutive infusions of 0, 3, 6 and 9 µg/kg fat free mass (FFM).min dobutamine, each dose for 30 min. The β_2 -adrenoceptor study involved three study periods during which 0, 50 and 100 ng/kg FFM.min salbutamol was given in combination with 1.2 µg/kg FFM.min atenolol (bolus: 50 µg/kg FFM), each dose for 45 min.

Measurements: Energy expenditure and respiratory exchange ratio (RER) were measured continuously by indirect calorimetry. At the end of each of each infusion period, blood samples for the determination of plasma non-esterified fatty acids (NEFA), glycerol, lactate, glucose, insulin, potassium, epinephrine, norepinephrine, dobutamine and salbutamol concentrations were taken and heart rate and blood pressure were measured.

Results: Energy expenditure, lipid oxidation, as measured by RER, and lipolysis, as measured by NEFA and glycerol concentrations, increased similarly in both groups during β_1 -adrenergic stimulation. During β_2 -adrenergic stimulation, the increases in energy expenditure and lipolysis were reduced in the obese group. Furthermore, lipid oxidation significantly increased in the normal weight group, but remained similar in the overweight group.

Conclusion: β_1 -Adrenoceptor-mediated metabolic processes were similar in obese and lean men, but β_2 -adrenoceptor-mediated increases in thermogenesis and lipid utilization were impaired in the obese.

Introduction

The sympathetic nervous system plays an important role in the regulation of thermogenesis and lipid utilization. Studies in which the endogenous catecholamines norepinephrine^{1,2} and epinephrine³⁻⁵ (both non-selective α - and β -adrenoceptor agonists) are infused show significant increases in energy expenditure, lipid oxidation and lipolysis. The role of the individual adrenoceptor subtypes in thermogenesis has also been studied. α -Adrenergic stimulation does not affect whole body thermogenesis,^{3,6} whereas non-selective β -adrenergic stimulation with isoprenaline significantly increases energy expenditure and lipid utilization.⁷ During only β_1 -adrenergic stimulation with dobutamine^{8,9} or only β_2 -adrenergic stimulation with salbutamol⁶ or terbutaline¹⁰, energy expenditure, lipid oxidation and lipolysis increase as well. In rats, β_3 -adrenergic stimulation also leads to significant increases in thermogenesis and lipid utilization.^{11,12} However, the rat β_3 -adrenoceptor differs pharmacologically from the human β_3 -adrenoceptor^{13,14} and consequently, the specific β_3 -adrenoceptor agonists used in rats are only weak agonists in humans. Until now, no highly selective β_3 -adrenoceptor agonist or antagonist is available for administration in humans.

Obese subjects may show an impaired response in energy expenditure during norepinephrine infusion,^{15,16} but similar responses as in lean subjects are also frequently found during norepinephrine,^{17,18} epinephrine^{5,19} and isoprenaline⁷ infusion. Others only found an impaired thermogenic response when very obese men were compared with very lean men²⁰ or only during overfeeding.¹⁸ More evident are the differences in lipid utilization between obese and lean subjects. During epinephrine^{5,19} or isoprenaline⁷ infusion, the increase in lipid oxidation is reduced in overweight men. Furthermore, their increases in plasma non-esterified fatty acids (NEFA) and glycerol concentrations are impaired during epinephrine^{5,21} or isoprenaline⁷ infusion. Only Katzeff *et al.*¹⁷ reported an opposite finding, e.g. that the increases in plasma glycerol and NEFA concentrations in response to norepinephrine infusion were proportional to the total fat mass of each individual and therefore were greater in the obese. Until now, it is unclear which β -adrenoceptor subtype is responsible for the found impaired responses in thermogenesis and lipid utilization.

The aim of the present studies was to elucidate the roles of the β_1 - and the β_2 -adrenoceptor in thermogenesis, lipid oxidation and lipolysis in obese and lean men.

Materials and methods

Subjects

Fourteen obese and 15 lean male volunteers participated in these studies. Six obese and 6 lean men participated in both studies within a time frame of 9 ± 2 months. Physical characteristics of the subjects, grouped per study, are summarized in table 5.1. All subjects were in good health as assessed by medical history and physical examination and were weight stable for at least 6 months. Furthermore, both obese and lean subjects spent no more than 2 h a week in organized sports activities. The study protocols were reviewed and approved by the Ethics Committee of Maastricht University and all subjects gave informed consent before participating in the tests.

Table 5.1 Physical characteristics of subjects participating in the β_1 - and the β_2 -adrenoceptor study.

	β_1 -Adrenoceptor study		β_2 -Adrenoceptor study	
	Obese	Lean	Obese	Lean
Body weight (kg)	103.1 (85.6-119.1) ***	73.3 (57.0-82.2)	102.3 (88.6-120.7) ***	73.7 (55.2-94.6)
Height (m)	1.78 (1.72-1.84)	1.76 (1.63-1.84)	1.76 (1.62-1.84)	1.77 (1.63-1.90)
BMI (kg/m ²)	32.4 (29.1-36.0) ***	23.7 (20.4-26.6)	33.2 (29.5-37.1) ***	23.4 (19.1-26.4)
Body fat (%)	31.4 (24.0-36.1) **	22.2 (14.0-31.0)	31.8 (22.0-43.9) ***	22.1 (13.2-26.4)
Age (y)	46 (39-51)	* 41 (34-50)	47 (40-50)	43 (34-50)

Values are mean (range) for 9 obese and 10 lean subjects in the β_1 -adrenoceptor study and 10 obese and 11 lean men in the β_2 -adrenoceptor study. BMI: body mass index. Unpaired t-test: obese vs lean: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Experimental design

Subjects were studied in the morning after an overnight fast. They came to the laboratory by car or by bus to minimize the amount of physical activity before the test. On arrival, a canula was inserted into a forearm vein of each arm. One canula was used for the infusion of drugs and one canula for the sampling of blood. Next, ventilated hood measurements were started with the subject in supine position and continued for the remainder of the experiment. At the end of each study period, a blood sample was taken. Room temperature was kept at 21-23 °C.

The β_1 -adrenoceptor study consisted of four study periods. After a 30 min baseline measurement, subjects received consecutive infusions of 3, 6 and 9 $\mu\text{g/kg}$ fat free mass (FFM).min dobutamine (Dobax[®], Byk, Zwanenburg, The Netherlands), each dose for 30 min. The β_2 -adrenoceptor study consisted of three study periods. At the start of the experiment, subjects received a priming dose of 50 $\mu\text{g/kg}$ FFM atenolol (β_1 -adrenoceptor antagonist) (Tenormin[®], Zeneca, Ridderkerk, The Netherlands) in 5 min, after which a continuous infusion of 1.2 $\mu\text{g/kg}$ FFM.min atenolol was started for the remainder of the experiment. After a 45 min 'baseline' measurement, subjects additionally received consecutive infusions of 50 and 100 ng/kg FFM.min salbutamol (Ventolin[®], GlaxoWellcome, Zeist, The Netherlands), each infusion for 45 min.

Clinical methods

Body density was determined by hydrostatic weighing with simultaneous lung volume measurement (Volugraph 2000, Mijnhardt, Bunnik, The Netherlands). Body composition was calculated according to the equation of Siri.²²

Whole body energy expenditure and respiratory exchange ratio (RER) were measured by indirect calorimetry, using an open-circuit ventilated hood system. In the β_1 -adrenoceptor study, a home-made system was used.²³ The volume of air drawn through the hood was measured by a dry-gas meter (Schlumberger, Dordrecht, The Netherlands) and the composition of the in- and outflowing air was analyzed by a paramagnetic O₂ analyzer (Servomex, Crowborough, UK) and an infrared CO₂ analyzer (Hartmann and Braun, Frankfurt, Germany). In the β_2 -adrenoceptor study, energy expenditure and RER were measured by an Oxycon

(Mijnhardt, Bunnik, The Netherlands). The airflow rate and the O_2 and CO_2 concentrations of the in- 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 coefficient of variation for O_2 consumption was 2.4% for the home-made system and 2.5% for the Oxycon; the coefficient of variation for CO_2 production was 3.1% for the home-made system and 2.0% for the Oxycon. Energy expenditure was calculated according to the formula proposed by Weir.²⁴ Energy expenditure and RER values were averaged over the last 10 min of each 30 (β_1) or 45 (β_2) min period during which steady state occurred. During the β_2 -adrenoceptor study, subjects collected their urine for nitrogen determination over a 12 h period before arriving at the laboratory. Nitrogen excretion was used to estimate protein oxidation at baseline and was assumed to be constant during the remainder of the test. For subjects that only participated in the β_1 -adrenoceptor study, the mean nitrogen excretion rate for the corresponding group in the β_2 -adrenoceptor study was used. After correction for protein oxidation, carbohydrate and lipid oxidation was calculated from O_2 consumption and CO_2 production as described by Ferrannini.²⁵

Heart rate was monitored continuously by conventional electrocardiography and the mean value over the last 10 min of each measuring period was used for further analysis. Blood pressure was measured by an automated blood pressure device (Tonoprint, Speidel & Keller, Jungingen, Germany) during the last 10 min of each 30 (β_1) or 45 (β_2) min interval. The means of four measurements per interval were used for further analysis.

Analytical methods

Blood samples for the determination of NEFA, glycerol, glucose, lactate and insulin were preserved in sodium-EDTA, samples for potassium in heparin and those for dobutamine, salbutamol, norepinephrine and epinephrine in heparin plus glutathione (1.5% w/v). Blood samples were immediately centrifuged for 10 min at 800 *g* at 4 °C. Plasma was transferred into microtest tubes, rapidly frozen in liquid nitrogen and stored at -70°C until further analysis.

Plasma NEFA concentration was measured with the NEFA C kit (99475409, WAKO, Neuss, Germany), plasma glycerol concentration was measured with a glycerol kit (148270, Boehringer, Mannheim, Germany), plasma glucose concentration was measured with a glucose kit (Unimate 5, 0736724, Roche Diagnostica, Basel, Switzerland) and plasma lactate concentration was measured by the method of Gutmann and Wahlefeld,²⁶ all on a Cobas-Fara centrifugal analyzer (Roche Diagnostica, Basel, Switzerland). Plasma insulin level was determined with a double antibody radio-immunoassay (Insulin RIA 100, Pharmacia, Uppsala, Sweden) and plasma potassium concentration by an ion-selective electrode (Salm & Kipp, Breukelen, The Netherlands). Plasma dobutamine, norepinephrine and epinephrine levels were determined by high performance liquid chromatography according to the method of Alberts *et al.*²⁷ Plasma salbutamol concentrations were measured by an in-house method (Analytico Medinet, Breda, The Netherlands) Salbutamol was first extracted from its matrix by means of a solid-phase extraction procedure. After derivatization with BSTFA (trimethylsilyl-trifluoroacetamide) its concentration was determined by using a capillary GC-MS method. Quantification was performed by monitoring the ion fragments at 456 *M/z* for salbutamol and 459 *M/z* for the internal standard D_3 -salbutamol (IS) and calculation of peak height ratio

analyte/IS amounts. The limit of quantification in plasma was 1.0 nmol/l, based on a 1 ml sample volume. The calibration range was between 1 and 40 nmol/l.

Standard samples with known concentrations were included in each run for quality control.

Data analysis

All data are presented as mean $\pm$ standard error of the mean (SEM). Data for energy expenditure were adjusted for FFM for group comparison.²⁸

The effect of β_1 - or β_2 -adrenergic stimulation between groups was analyzed with two-way repeated measurements ANOVA. Post hoc testing was done with Student's unpaired t-test. A P-value < 0.05 was regarded as statistically significant.

Results

β_1 -adrenoceptor study

Baseline energy expenditure was significantly higher in obese compared to lean men (5.49 ± 0.21 vs 4.61 ± 0.18 kJ/min, $P < 0.01$), but after adjustment for FFM, it was comparable between groups (obese vs lean: 5.19 ± 0.25 vs 5.15 ± 0.14 kJ/min adjusted for FFM, NS). During β_1 -adrenergic stimulation, energy expenditure increased significantly (figure 5.1). RER was similar at baseline between obese and lean men (0.799 ± 0.013 vs 0.797 ± 0.011 , NS). RER significantly decreased during β_1 -adrenergic stimulation. Lipid and carbohydrate oxidation were comparable in obese and lean subjects at baseline (lipid oxidation: 76 ± 7 vs 66 ± 6 mg/min, NS; carbohydrate oxidation: 93 ± 19 vs 77 ± 14 mg/min, NS). Lipid oxidation significantly increased and carbohydrate oxidation significantly decreased during β_1 -adrenergic stimulation. The changes in energy expenditure, RER, lipid oxidation and carbohydrate oxidation were similar in obese and lean men (figure 5.1).

At baseline, plasma NEFA and glycerol levels were similar in obese and lean men (NEFA: 542 ± 60 vs 409 ± 46 $\mu\text{mol/l}$, NS; glycerol: 77.7 ± 8.2 vs 62.6 ± 8.6 $\mu\text{mol/l}$, NS). Both groups showed similar dose-related increases in plasma NEFA and glycerol levels (figure 5.2). Baseline glucose and insulin concentrations were significantly higher in the obese as compared to the lean group (glucose: $P < 0.05$, insulin: $P < 0.01$) (table 5.2). Plasma glucose levels significantly decreased and plasma insulin levels significantly increased during β_1 -adrenergic stimulation with dobutamine. The changes in these parameters compared to baseline were not significantly different between groups. At baseline, plasma lactate and potassium levels were similar in obese and lean men. During β_1 -adrenergic stimulation, plasma lactate levels remained similar, whereas plasma potassium levels showed some variation, but no dose-dependent changes in both groups (table 5.2).

Plasma dobutamine levels significantly increased to similar concentrations in obese and lean men (figure 5.3). Baseline norepinephrine and epinephrine levels were comparable between overweight and normal weight subjects (norepinephrine: 1.59 ± 0.35 vs 1.47 ± 0.27 nmol/l, epinephrine: 0.19 ± 0.04 vs 0.22 ± 0.03 nmol/l, both NS) and significantly decreased in both groups during β_1 -adrenergic stimulation (figure 5.3).

Baseline values for heart rate and systolic blood pressure were not significantly different between groups (table 5.3), but diastolic blood pressure was significantly higher in obese men ($P < 0.01$). Heart rate and systolic blood pressure significantly increased and diastolic blood pressure significantly decreased in both groups during β_1 -adrenergic stimulation with dobutamine. The changes in heart rate and in systolic and diastolic blood pressure were comparable in both groups (table 5.3).

β_2 -adrenoceptor study

Baseline energy expenditure was similar in obese and lean men (5.13 ± 0.16 vs 4.97 ± 0.09 kJ/min adjusted for FFM, NS). During β_2 -adrenergic stimulation, adjusted energy expenditure significantly increased. However, the increase in energy expenditure was significantly lower in the obese compared to the lean group (ANOVA for energy expenditure $\times$ group: $P < 0.05$) (figure 5.4). At baseline, RER was similar in obese and lean men (0.838 ± 0.011 vs 0.825 ± 0.008 , NS). RER significantly decreased during β_2 -adrenergic stimulation, but the decrease was significantly higher in the lean group (ANOVA for RER $\times$ group: $P < 0.05$) (figure 5.4). Baseline lipid and carbohydrate oxidation were similar in obese and lean subjects (lipid oxidation: 55 ± 6 vs 42 ± 3 mg/min, NS; carbohydrate oxidation: 143 ± 17 vs 116 ± 9 mg/min,

Figure 5.1 Changes in energy expenditure adjusted for fat free mass, respiratory exchange ratio and lipid and carbohydrate oxidation during β_1 -adrenergic stimulation with dobutamine in 9 obese (●) and 10 lean (Δ) men. Values are mean $\pm$ SEM. One-way repeated measurements ANOVA: ### $P < 0.001$.

Table 5.2 Plasma concentrations of glucose, insulin, lactate and potassium during β_1 -adrenergic stimulation with dobutamine in obese and lean men.

Parameter	Group	Dobutamine ($\mu\text{g/kg FFM.min}$)				ANOVA	
		0	3	6	9	Parameter	Par x Group
Glucose (mmol/l)	Obese	5.56 $\pm$ 0.16	5.31 $\pm$ 0.21	5.16 $\pm$ 0.18	5.01 $\pm$ 0.18	P<0.001	NS
	Lean	5.04 $\pm$ 0.15*	4.86 $\pm$ 0.15	4.74 $\pm$ 0.12	4.67 $\pm$ 0.11		
Insulin (mU/l)	Obese	14.0 $\pm$ 2.7	19.4 $\pm$ 4.7	21.0 $\pm$ 4.5	22.7 $\pm$ 5.4	P<0.001	NS
	Lean	6.1 $\pm$ 0.6**	7.8 $\pm$ 1.0*	9.3 $\pm$ 1.2*	10.5 $\pm$ 1.2*		
Lactate (mmol/l)	Obese	1.28 $\pm$ 0.16	1.28 $\pm$ 0.17	1.27 $\pm$ 0.17	1.13 $\pm$ 0.10	NS	NS
	Lean	0.93 $\pm$ 0.14	0.83 $\pm$ 0.07*	0.81 $\pm$ 0.06*	0.74 $\pm$ 0.03**		
Potassium (mmol/l)	Obese	4.22 $\pm$ 0.11	4.26 $\pm$ 0.09	4.17 $\pm$ 0.09	4.16 $\pm$ 0.08	P<0.05	NS
	Lean	4.07 $\pm$ 0.08	4.16 $\pm$ 0.09	4.18 $\pm$ 0.08	4.02 $\pm$ 0.07		

Values are mean $\pm$ SEM for 9 obese subjects and 10 lean subjects. Unpaired t-test: obese vs lean: *P<0.05, **P<0.01.

Table 5.3 Heart rate and systolic and diastolic blood pressure (BP) during β_1 -adrenergic stimulation with dobutamine in obese and lean men.

Parameter	Group	Dobutamine ($\mu\text{g/kg FFM.min}$)				ANOVA	
		0	3	6	9	Treatment	Par x Group
Heart rate (beats/min)	Obese	66 $\pm$ 3	65 $\pm$ 4	68 $\pm$ 4	76 $\pm$ 5	P<0.001	NS
	Lean	59 $\pm$ 2	59 $\pm$ 2	64 $\pm$ 2	77 $\pm$ 5		
Systolic BP (mmHg)	Obese	126 $\pm$ 4	140 $\pm$ 6	150 $\pm$ 7	157 $\pm$ 6	P<0.001	NS
	Lean	122 $\pm$ 4	142 $\pm$ 5	153 $\pm$ 4	160 $\pm$ 4		
Diastolic BP (mmHg)	Obese	95 $\pm$ 2	89 $\pm$ 3	82 $\pm$ 3	80 $\pm$ 3	P<0.001	NS
	Lean	84 $\pm$ 3*	80 $\pm$ 3	76 $\pm$ 4	75 $\pm$ 5		

Values are mean $\pm$ SEM for 9 obese and 10 lean subjects. Unpaired t-test: obese vs lean: *P<0.05.

NS). Lipid oxidation significantly increased during β_2 -adrenergic stimulation, but this increase was significantly higher in the lean group (ANOVA for lipid oxidation x group: $P=0.05$). Carbohydrate oxidation rates significantly decreased (ANOVA for treatment: $P<0.05$) during β_2 -adrenergic stimulation, but did not differ significantly between groups (figure 5.4).

At baseline, plasma NEFA levels were similar in obese and lean men (443 ± 21 vs 395 ± 34 $\mu\text{mol/l}$, NS) (figure 5.2). Baseline glycerol levels were significantly higher in the overweight compared to the normal weight group (76.5 ± 4.3 vs 61.7 ± 4.8 $\mu\text{mol/l}$, $P<0.05$). During β_2 -adrenergic stimulation with salbutamol, plasma NEFA and glycerol levels increased significantly more in the lean compared to the obese group (ANOVA for group x treatment, NEFA: $P<0.01$, glycerol: $P<0.05$) (figure 5.2). Plasma glucose and insulin levels were significantly higher in the obese group at baseline (table 5.4). Plasma glucose levels remained similar in the overweight group, but increased significantly in the normal weight group during β_2 -adrenergic stimulation. Plasma insulin levels increased significantly more in the obese as compared to the lean group during salbutamol infusion. Baseline lactate and potassium concentrations were similar in both groups. Plasma lactate levels significantly increased

Figure 5.2 Plasma non-esterified fatty acids (NEFA) and glycerol concentrations during β_1 -adrenergic stimulation with dobutamine in 9 obese (●) and 10 lean (Δ) men (left panels) and during β_2 -adrenergic stimulation with salbutamol in combination with atenolol in 10 obese (●) and 11 lean (Δ) men (right panels). Values are mean $\pm$ SEM. One-way repeated measurements ANOVA: *** $P<0.001$. Unpaired t-test: obese vs lean: * $P<0.05$, *** $P<0.001$.

Figure 5.3 Plasma dobutamine (left panel) and salbutamol (right panel) concentrations and plasma norepinephrine and epinephrine concentrations during β_1 -adrenergic stimulation with dobutamine in 9 obese (●) and 10 lean (Δ) men (left panels) and during β_2 -adrenergic stimulation with salbutamol in combination with atenolol in 10 obese (●) and 11 lean (Δ) men (right panels). Values are mean $\pm$ SEM. One-way repeated measurements ANOVA: # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$. Unpaired t-test: obese vs lean: * $P < 0.05$.

and plasma potassium levels significantly decreased during β_2 -adrenergic stimulation, but remained comparable between groups (table 5.5).

Plasma salbutamol concentrations increased to a similar level in obese and lean men during both infusion periods (figure 5.3). Baseline norepinephrine levels were comparable between overweight and normal weight subjects (2.57 ± 0.16 vs 2.36 ± 0.16 nmol/l, NS), but baseline epinephrine levels were significantly higher in the lean group (0.13 ± 0.02 vs 0.22 ± 0.03 nmol/l, $P < 0.01$). Plasma norepinephrine levels increased similarly in both groups during β_2 -adrenergic stimulation. Plasma epinephrine levels decreased significantly in both groups during β_2 -adrenergic stimulation, but the decrease was significantly higher in the lean group (figure 5.3).

Baseline values for heart rate and systolic and diastolic blood pressure were not significantly different between obese and lean men (table 5.5). Heart rate significantly increased, systolic blood pressure remained similar and diastolic blood pressure significantly decreased in both groups during β_2 -adrenergic stimulation with salbutamol. The changes in heart rate and systolic and diastolic blood pressure were similar in obese and lean men (table 5.5).

Discussion

The aim of the present studies was to examine the roles of the β_1 - and the β_2 -adrenoceptor in thermogenesis and lipid utilization in obese and lean men. During β_1 -adrenergic stimulation with dobutamine, no differences were found in the changes in energy expenditure and lipid utilization between groups. During β_2 -adrenergic stimulation with salbutamol, obese subjects had a reduced increase in energy expenditure, a reduced decrease in RER, suggesting a blunted increase in lipid oxidation, and a reduced increase in plasma NEFA and glycerol levels, suggesting a reduced lipolytic response. Even when comparing similar increases in thermogenesis in the lean between studies, the accompanying changes in expenditure, RER and plasma NEFA and glycerol concentrations in the obese were blunted during β_2 -adrenergic stimulation. This is in line with other studies,^{5,7,19} who found similar impaired responses during sympathetic activation in the obese.

The interpretation of the data from our study highly depends on the selectivity of the β -adrenoceptor agonists used. An earlier study from our group⁹ showed that dobutamine induced β_1 -adrenoceptor-specific changes in thermogenesis and lipid utilization in dosages ≤ 10 $\mu\text{g/kg}$ body weight (BW).min. The maximum dose we used was 9 $\mu\text{g/kg}$ FFM.min, which is comparable with 7.5 $\mu\text{g/kg}$ BW.min, and thus lies within the range of β_1 -adrenoceptor specificity. Our earlier study⁹ also showed that the β_2 -adrenoceptor agonist salbutamol in a concentration of 85 ng/kg BW.min (or 100 ng/kg FFM.min) also induced β_1 -adrenoceptor-specific changes in lipid utilization. Addition of the β_1 -adrenoceptor antagonist atenolol prevented simultaneous β_1 -adrenergic stimulation, but did not affect β_2 -adrenoceptor-specific changes. Therefore, in the current study salbutamol was given in combination with atenolol to investigate β_2 -adrenoceptor specific changes in thermogenesis and lipid utilization.

Our study suggests that it is the β_2 -adrenoceptor which is responsible for the impaired responses in thermogenesis, lipid oxidation and lipolysis in the obese *in vivo*. In *in vitro* studies, similar results are found in relation to lipolysis. Glycerol release from subcutaneous

Table 5.4 Plasma concentrations of glucose, insulin, lactate and potassium during β_2 -adrenergic stimulation with salbutamol in combination with atenolol in obese and lean men.

Parameter	Group	Salbutamol (ng/kg FFM.min)			ANOVA	
		0	50	100	Treatment	Par x Group
Glucose (mmol/l)	Obese	5.64 $\pm$ 0.18	5.56 $\pm$ 0.17	5.67 $\pm$ 0.14	P<0.01	P<0.05
	Lean	4.89 $\pm$ 0.12 **	4.96 $\pm$ 0.15 *	5.17 $\pm$ 0.15 *		
Insulin (mU/l)	Obese	11.8 $\pm$ 2.4	17.2 $\pm$ 4.0	17.9 $\pm$ 3.3	P<0.001	P<0.05
	Lean	5.0 $\pm$ 0.4 **	6.8 $\pm$ 0.8 *	8.5 $\pm$ 1.1 *		
Lactate (mmol/l)	Obese	1.06 $\pm$ 0.11	1.12 $\pm$ 0.11	1.27 $\pm$ 0.10	P<0.001	NS
	Lean	0.86 $\pm$ 0.13	0.89 $\pm$ 0.08	1.03 $\pm$ 0.07		
Potassium (mmol/l)	Obese	4.32 $\pm$ 0.01	4.32 $\pm$ 0.07	4.05 $\pm$ 0.07	P<0.001	NS
	Lean	4.21 $\pm$ 0.07	4.29 $\pm$ 0.08	4.03 $\pm$ 0.08		

Values are mean $\pm$ SEM for 10 obese and 11 lean subjects. Unpaired t-test: obese vs lean: * P<0.05, ** P<0.01.

Table 5.5 Heart rate and systolic and diastolic blood pressure (BP) during β_2 -adrenergic stimulation with salbutamol in combination with atenolol in obese and lean men.

Parameter	Group	Salbutamol (ng/kg FFM.min)			ANOVA	
		0	50	100	Treatment	Par x Group
Heart rate (beats/min)	Obese	58 $\pm$ 2	65 $\pm$ 2	74 $\pm$ 3	P<0.001	NS
	Lean	53 $\pm$ 2	59 $\pm$ 2	67 $\pm$ 3		
Systolic BP (mmHg)	Obese	121 $\pm$ 4	119 $\pm$ 4	122 $\pm$ 5	NS	NS
	Lean	113 $\pm$ 2	117 $\pm$ 3	118 $\pm$ 2		
Diastolic BP (mmHg)	Obese	90 $\pm$ 4	85 $\pm$ 4	83 $\pm$ 4	P<0.001	NS
	Lean	82 $\pm$ 3	80 $\pm$ 3	75 $\pm$ 2		

Values are mean $\pm$ SEM for 10 obese and 11 lean subjects.

abdominal fat cells from normal weight and overweight women was similar after incubation with dobutamine, but after incubation with isoprenaline or terbutaline, glycerol release was reduced in fat cells from the obese. This appeared to be due to a significant reduction in cell surface density of β_2 -adrenoceptors, although mRNA levels were similar in both groups.²⁹ In another study, lean subjects with a low isoprenaline sensitivity, as measured by *in vitro* subcutaneous abdominal fat cell lipolysis, appeared to have a lower β_2 -adrenoceptor number and mRNA level compared to lean subjects with a high isoprenaline sensitivity, whereas β_1 -adrenoceptor number and mRNA levels were similar in both groups.³⁰ Both studies suggest that the β_2 -adrenoceptor is responsible for the reduced β -adrenoceptor-mediated increase in lipolysis, which is in line with our findings.

Further evidence for a role of the β_2 -adrenoceptor in the etiology of obesity is provided by two recently found polymorphisms in the β_2 -adrenoceptor that are associated with obesity. The Arg16Gly polymorphism in the β_2 -adrenoceptor is associated with obesity in Japanese women.³¹ In a group of Swedish women, this mutation is not associated with obesity, but fat cells from women homozygous for Arg16 showed a 5-fold lower agonist sensitivity for β_2 -adrenoceptors than women heterozygous or homozygous for Gly16.³² The Gln27Glu poly-

Figure 5.4 Energy expenditure, lipid oxidation and carbohydrate oxidation adjusted for fat free mass and respiratory exchange ratio during β_2 -adrenergic stimulation with salbutamol in combination with atenolol in 10 obese (●) and 11 lean (Δ) men. Values are mean $\pm$ SEM. One-way repeated measurements ANOVA: ** $P < 0.01$, *** $P < 0.001$. Unpaired t-test: obese vs lean: * $P < 0.05$.

morphism is associated with obesity in Japanese males and females.^{31,33} Swedish women homozygous for Glu27 had an average fat mass excess of 20 kg and ~ 50% larger fat cells as compared to women homozygous for Gln27. However, no significant association with changes in β_2 -adrenoceptor function was observed as assessed by *in vitro* fat cell lipolysis experiments.³² Obesity in Swedish males tends to be negatively associated with the Gln27Glu polymorphism.³⁴ Since we did not determine β_2 -adrenoceptor polymorphisms, it is unknown whether the found impaired responses in thermogenesis and lipid utilization in our obese group are associated with one or both of the above mentioned polymorphisms. Until now, no associations between polymorphisms in the β_1 -adrenoceptor and obesity have been reported.

The reduced increases in thermogenesis and lipid oxidation during β_2 -adrenergic stimulation in the obese might also be explained by the reduced increase in NEFA in the blood. The amount of NEFA presented to skeletal muscle was therefore reduced, which may have resulted in a smaller increase in lipid oxidation and thermogenesis. As shown in figure 5.5, there was a clear relationship between the increases in plasma NEFA concentration and the increases in energy expenditure and lipid oxidation during β_1 - and β_2 -adrenergic stimulation. Furthermore, we recently reported that for a certain increase in plasma NEFA concentration by lipid heparin infusion, similar increases in thermogenesis and lipid oxidation are found in obese and lean men.³⁵ These data suggest that not only β_2 -adrenergic stimulation, but also NEFA availability might be related to the blunted responses in thermogenesis and lipid oxidation. Other studies reported not only impaired responses in adipose tissue metabolism, but also in skeletal muscle metabolism, where thermogenesis and lipid oxidation are presumed to be predominantly localized.³⁶ Blaak *et al.*⁷ showed that although plasma NEFA levels increased significantly during non-selective β -adrenergic stimulation, no net uptake of NEFA in skeletal muscle occurred in the obese. Moreover, others found that obese women have a decreased oxidative capacity and increased glycolytic and anaerobic capacities, as

measured by the activities of several key enzymes in skeletal muscle biopsies.^{37,38} This suggests that lipid oxidation and energy expenditure are impaired in the obese independent from NEFA availability. Our β_1 -adrenoceptor study and the referred study with the lipid heparin infusion³⁵ show that similar increases in thermogenesis and lipid oxidation occur in obese and lean men for a certain increase in plasma NEFA concentration and therefore do not support these findings.

The reduced increase in plasma NEFA and glycerol concentration and the consequent reduced increases in lipid oxidation and thermogenesis during β_2 -adrenergic stimulation might not only be explained by a defect in the β_2 -adrenoceptor or the pathways it mediates, as stated above, but also by the slightly higher increase in insulin concentration in the obese, for insulin inhibits lipolysis. But since the obese group was insulin resistant according to their high baseline insulin levels, the impact of this higher increase is difficult to interpret. Comparing the β_1 - with the β_2 -adrenoceptor study, the increases in plasma insulin level were almost identical between studies (obese vs lean, β_1 -adrenoceptor study: 8.7 ± 3.5 vs 4.4 ± 0.8 mU/l; β_2 -adrenoceptor study: 6.0 ± 1.0 vs 3.5 ± 0.8 mU/l), whereas the increases in NEFA and glycerol levels, lipid oxidation and thermogenesis were only impaired in the β_2 -adrenoceptor study. Furthermore in *in vitro* lipolysis tests, β_2 -adrenoceptor-mediated lipolysis was reduced in fat cells from the obese, although no insulin was present in the incubation media.^{29,30} These data suggest that the impaired increases in plasma NEFA and glycerol concentration were not due to the slightly higher increase in plasma insulin concentration in the obese during β_2 -adrenergic stimulation. However, since changes in insulin concentration and not in insulin action were measured, repeating the experiment during a hyperinsulinaemic clamp can only provide direct evidence for a role of insulin in the blunted responses in the obese. Two other studies investigated the role of insulin in epinephrine-induced thermogenesis. One study showed that epinephrine induced energy expenditure independent from insulin concentrations,³⁹ whereas the other study found an inhibitory effect of insulin.⁴⁰

Aging is also known to reduce the sensitivity for catecholamines and thus for β -adrenoceptor agonists.^{41,42} In our β_2 -adrenoceptor study, obese and lean subjects were of similar age, but in the β_1 -adrenoceptor study, the obese group was slightly, but significantly older than the lean one. However, since our groups differed only 5 years in age, whereas subjects in studies on the effect of aging commonly differ more than 30 years of age, we believe that the difference in catecholamine sensitivity between our subjects was only minor and therefore did not influence the interpretation of our data.

The impaired responses to β_2 -adrenergic stimulation may be caused by differences in norepinephrine kinetics. Studies with tritiated norepinephrine have shown that norepinephrine appearance rates are similar^{43,44} or higher^{45,46} and norepinephrine clearance rates are similar^{17,45,46} or lower¹⁸ in subjects with a greater fat mass. This suggests that basal sympathetic nervous system activity may be chronically increased in the obese. As a consequence, β -adrenoceptors may become desensitized and/or down regulated resulting in a reduced sympathetic nervous system response during additional β -adrenergic stimulation, as shown in this and other studies.^{5,7,15,16,19-21} With regard to our study, it is unclear why this desensitization and/or down regulation would only affect the β_2 - and not the β_1 -adrenoceptor.

The question remains whether the impaired responses during β_2 -adrenergic stimulation are a cause or a consequence of obesity. Blaak *et al.*⁴⁷ showed that β -adrenoceptor-mediated thermogenesis tended to increase after weight loss. This suggests the impaired sympathetic nervous system response is a consequence of the obese state. On the other hand, Astrup *et al.*⁴⁸ showed that glucose-induced increases in energy expenditure and norepinephrine concentrations improved in obese subjects after 30 kg weight loss, but were still lower than that of control subjects. Furthermore, Blaak *et al.*⁴⁷ showed that β -adrenoceptor-mediated increases in arterial NEFA concentration and muscle NEFA uptake remained impaired after weight reduction. This suggests that a defective sympathetic nervous system may rather be a primary factor leading to the development of obesity than a secondary factor resulting from the obese state.

In conclusion, our studies suggest that β_1 -adrenoceptor-mediated thermogenesis and lipid utilization are similar in obese and lean men, but β_2 -adrenoceptor-mediated increases in energy expenditure, lipid oxidation and lipolysis are impaired in the obese.

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THE EFFECT OF AN INCREASED NEFA CONCENTRATION ON THERMOGENESIS AND SUBSTRATE OXIDATION IN OBESE AND LEAN MEN

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SLH Schiffelers, WHM Saris and MA van Baak

Nutrition Toxicology and Environment Research Institute Maastricht,
Department of Human Biology, Maastricht University,
Maastricht, The Netherlands

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Abstract

Aim: To examine whether a certain increase in plasma non-esterified fatty acids (NEFA) concentration leads to similar increases in lipid oxidation and energy expenditure in obese and lean men.

Subjects: Eleven obese and 13 lean men with a mean body mass index (BMI) of 34.2 kg/m^2 (range: $29.3\text{--}40.6 \text{ kg/m}^2$) and 23.9 kg/m^2 (range: $20.3\text{--}26.4 \text{ kg/m}^2$) and a mean age of 46 y (range: 40–50 y) and 43 y (range: 35–50 y), respectively.

Design: The study protocol consisted of a 30 min baseline period after which subjects received a bolus of 1000 IU heparin. Then, consecutive 30 min infusions of 4.9, 9.8 and $19.6 \mu\text{l/kg}$ fat free mass.min of a lipid heparin mixture were started.

Measurements: Energy expenditure, respiratory exchange ratio (RER) and carbohydrate and lipid oxidation were continuously measured by indirect calorimetry. At the end of each infusion period, a blood sample for the determination of plasma NEFA, glycerol, insulin, β -hydroxybutyrate, norepinephrine and epinephrine concentrations was taken.

Results: At baseline, plasma NEFA levels were similar in both groups. Lipid heparin infusion increased plasma NEFA concentration by 301 ± 47 and $332 \pm 27 \mu\text{mol/l}$ in obese and lean men. Energy expenditure increased similarly in obese and lean men (0.34 ± 0.08 vs $0.40 \pm 0.08 \text{ kJ/min}$, NS) during lipid heparin infusion, whereas RER decreased similarly in both groups. Lipid oxidation rates were comparable at baseline and increased similarly in obese and lean men (19 ± 5 vs $13 \pm 4 \text{ mg/min}$, NS). Baseline plasma insulin concentrations were higher in the obese, but did not change during lipid heparin infusion. Plasma β -hydroxybutyrate concentrations were similar at baseline, but increased significantly less in the obese during lipid heparin infusion. Plasma norepinephrine and epinephrine concentrations did not significantly differ between groups at baseline. During lipid heparin infusion, plasma norepinephrine levels decreased significantly and plasma epinephrine levels remained unchanged in both groups.

Conclusion: A certain increase in plasma NEFA concentration leads to similar increases in lipid oxidation and energy expenditure in obese and lean men. The accumulation of fat in obese subjects may therefore more likely be due to a defect in adipose tissue lipolysis than a defect in lipid oxidation.

Introduction

Energy expenditure increases after the ingestion or infusion of nutrients. Activation of the sympathetic nervous system (SNS) may, at least partly, contribute to this increase in thermogenesis,¹⁻³ although this finding is not consistent.⁴ Previous studies have shown that mainly β_1 - and β_2 -adrenoceptors are involved in sympathetically mediated thermogenesis.^{5,6} During selective β_1 -adrenergic stimulation, lipolysis, lipid oxidation and energy expenditure increase.^{7,8} Non-esterified fatty acids (NEFA), needed for lipid oxidation, are released from the adipose tissue by stimulating its β_1 -adrenoceptors. The increase in lipid oxidation and thermogenesis is assumed to be localized predominantly in skeletal muscle,^{9,10} but this tissue contains mainly β_2 -adrenoceptors and presumably no β_1 -adrenoceptors.¹¹ β_1 -Adrenergic stimulation is therefore not likely to increase lipid oxidation by direct stimulation of skeletal muscle. Another possibility is that the availability of NEFA in the blood may induce the increase in lipid oxidation and energy expenditure.

Furthermore, it is suggested that the thermogenic effect of food is reduced in obese subjects, although the results are inconsistent (for review see De Jonge *et al.*¹²). A reduction in diet-induced thermogenesis in the obese might be explained by their reduced thermogenic response during SNS stimulation.^{13,14} Blaak *et al.*¹⁰ showed that obese men have an impaired response in lipolysis and lipid oxidation during isoprenaline (non-selective β -agonist) infusion. If the availability of NEFA in blood is a limiting factor for thermogenesis, the reduced lipolytic response in obese subjects could explain their reduced increases in lipid oxidation and thermogenesis.

On the other hand, thermogenesis and lipid oxidation could be impaired due to a defect in skeletal muscle metabolism. Colberg *et al.*¹⁵ showed that women with visceral obesity have reduced NEFA utilization in muscle in the postabsorptive state. Furthermore, Colberg *et al.*¹⁵ and Simoneau *et al.*¹⁶ found that obese women have a decreased oxidative capacity and increased glycolytic and anaerobic capacities in skeletal muscle. This may suggest that not NEFA availability in blood, but rather the oxidative enzymes in skeletal muscle may be the limiting factor for the increase in lipid oxidation and thermogenesis.

The aim of the present study was to examine whether a certain increase in plasma NEFA concentration leads to similar increases in lipid oxidation and energy expenditure in obese and lean men.

Material and methods

Subjects

Eleven obese and 13 lean male volunteers participated in this study. Physical characteristics of the subjects are summarized in table 6.1. All subjects were in good health as assessed by medical history and physical examination. Furthermore, both lean and obese subjects spent no more than 2 hours a week in organized sports activities. The study protocol was reviewed and approved by the Ethics Committee of Maastricht University and all subjects gave informed consent before participating in the study.

Table 6.1 Subject characteristics.

Parameter	Obese			Lean	
Body weight (kg)	106.9	(94.5 - 117.1)	***	75.2	(59.5 - 79.3)
Height (m)	1.77	(1.62 - 1.87)		1.77	(1.63 - 1.90)
Body mass index (kg/m ²)	34.2	(29.3 - 40.6)	***	23.9	(20.3 - 26.4)
Body fat (%)	32.4	(23.6 - 41.6)	***	19.1	(10.3 - 28.5)
Age (y)	46	(40 - 50)		43	(35 - 50)

Values are mean (range) for 11 obese and 13 lean subjects. Unpaired t-test: obese vs lean: *** $P < 0.001$.

Experimental design

Subjects arrived at the laboratory at 8:00 AM after an overnight fast. They came by car or by bus to minimize the amount of physical activity before the test. On arrival, one canula was inserted into a forearm vein to infuse a lipid heparin mixture and to sample venous blood. A second canula was inserted into a dorsal hand vein of the contralateral arm. This hand was kept in a hotbox with a temperature of 60 °C for the sampling of arterialized blood. Ventilated hood measurements were started with the subject in supine position and room temperature was kept between 21-23 °C.

After a 30 min baseline measurement, a bolus of 1000 IE heparin (Leo Pharmaceutical Products, Weesp, The Netherlands) was given after which a continuous infusion of increasing doses of 4.9, 9.8 and 19.6 µl/kg fat free mass (FFM).min of a lipid heparin mixture (Intralipid 20%®, Pharmacia, Woerden, The Netherlands) (1000 IE heparin per 100 ml Intralipid) was started, each dose given for 30 min. At the end of each 30 min period, a blood sample was taken. In a pilot study, blood samples were taken after 20, 25 and 30 min in each infusion period. The coefficient of variation (CV) for plasma NEFA concentration within each set of three samples was 5%. Therefore, it was assumed that steady state was achieved in plasma NEFA concentrations at the end of each infusion period.

Clinical methods

Body density was determined by hydrostatic weighing with simultaneous lung volume measurement (Volugraph 2000, Mijnhardt, Bunnik, The Netherlands). Body composition was calculated according to the equation of Siri.¹⁷

Whole body energy expenditure and respiratory exchange ratio (RER) were measured by an open-circuit ventilated hood system (Oxycon, Mijnhardt, Bunnik, The Netherlands). O₂ consumption (CV: 3.0%) and CO₂ production (CV: 1.7%) values were averaged over the last 10 min of each 30 min period. Energy expenditure was calculated according to the formula proposed by Weir.¹⁸ Carbohydrate and lipid oxidation rates were calculated as described by Ferrannini,¹⁹ assuming that protein oxidation accounted for 15% of total baseline energy expenditure and remained constant during the remainder of the test.

Analytical methods

Arterialized blood samples for the determination of NEFA, glycerol and insulin concentrations were preserved in sodium-EDTA. Venous samples for the determination of norepinephrine

and epinephrine concentrations were preserved in heparin plus glutathione (1.5% w/v). Blood samples were immediately centrifuged for 10 min at $800 \times g$ at 4°C . Plasma was transferred into microtest tubes, rapidly frozen in liquid nitrogen and stored at -70°C until further analysis. Plasma NEFA concentrations were measured with the NEFA C kit (9947-5409, WAKO, Neuss, Germany), plasma glycerol concentrations were measured with a glycerol kit (148270, Boehringer, Mannheim, Germany) and plasma β -hydroxybutyrate concentrations were measured according to the method of Moore *et al.*,²⁰ all on a Cobas-Fara centrifugal analyzer (Roche Diagnostica, Basel, Switzerland). Plasma insulin levels were determined with a double-antibody radio immunoassay (Insulin RIA 100, Pharmacia, Uppsala, Sweden) and plasma norepinephrine and epinephrine levels by high performance liquid chromatography.²¹ Standard samples with known concentrations were included in each run for quality control.

Data analysis

All data are presented as mean $\pm$ standard error of the mean (SEM). Data for energy expenditure were adjusted for FFM for group comparison.²²

The effect of lipid heparin infusion between obese and lean subjects was analyzed with two-way repeated measurements ANOVA. Post hoc testing was done with an unpaired t-test. Effects within a group were analyzed with one-way repeated measurements ANOVA. A P-value < 0.05 was regarded as statistically significant.

Results

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At baseline, plasma NEFA and glycerol levels were similar in both groups. Lipid heparin infusion significantly increased NEFA and glycerol concentrations (figure 6.1). These increases were not significantly different between groups (obese vs lean, Δ NEFA: 301 ± 47 vs $332 \pm 27 \mu\text{mol/l}$, NS; Δ glycerol: 170 ± 8 vs $151 \pm 11 \mu\text{mol/l}$, NS).

Figure 6.1 Plasma non-esterified fatty acids (NEFA) and glycerol concentrations before and during the infusion of a lipid heparin mixture in 11 obese (●) and 13 lean (Δ) men. Values are mean $\pm$ SEM. One-way repeated measurements ANOVA: ### P <0.001 .

Table 6.2 Energy expenditure and lipid and carbohydrate oxidation rates at rest and during lipid heparin infusion in obese and lean men.

Parameter	Group	Lipid heparin mixture ($\mu\text{mol/kg FFM.min}$)				ANOVA	
		0	4.9	9.8	19.6	Parameter	Par x Group
Energy expenditure (kJ/min)	Obese	5.97 ± 0.19	6.08 ± 0.19	6.07 ± 0.20	6.31 ± 0.19	P<0.001	NS
	Lean	$4.80 \pm 0.14^{***}$	$4.91 \pm 0.17^{***}$	$4.93 \pm 0.14^{***}$	$5.20 \pm 0.14^{***}$		
Lipid oxidation (mg/min)	Obese	64 ± 7	73 ± 9	71 ± 9	83 ± 6	P<0.001	NS
	Lean	54 ± 3	58 ± 3	56 ± 3	68 ± 4		
Carbohydrate oxidation (mg/min)	Obese	150 ± 20	133 ± 22	138 ± 23	121 ± 16	P<0.05	NS
	Lean	114 ± 9	113 ± 10	119 ± 9	106 ± 10		

Values are mean $\pm$ SEM for 11 obese and 13 lean men. Unpaired t-test: obese vs lean: *** P<0.001.

Table 6.3 Plasma insulin, β -hydroxybutyrate, norepinephrine and epinephrine concentrations at rest and during lipid heparin infusion in obese and lean men.

Parameter	Group	Lipid heparin mixture ($\mu\text{mol/kg FFM.min}$)				ANOVA	
		0	4.9	9.8	19.6	Parameter	Par x Group
Insulin (mU/l)	Obese	19.4 ± 3.5	19.0 ± 3.2	19.3 ± 3.3	19.7 ± 3.7	NS	NS
	Lean	$7.2 \pm 0.8^{***}$	$7.2 \pm 0.9^{***}$	$7.0 \pm 0.8^{***}$	$6.9 \pm 0.7^{***}$		
β -Hydroxybutyrate ($\mu\text{mol/l}$)	Obese	104 ± 12	96 ± 35	98 ± 22	$150 \pm 34^*$	P<0.001	P<0.05
	Lean	113 ± 20	140 ± 26	174 ± 35	277 ± 43		
Norepinephrine (nmol/l)	Obese	2.26 ± 0.24	2.03 ± 0.23	2.00 ± 0.29	1.90 ± 0.25	P<0.01	NS
	Lean	2.09 ± 0.22	1.91 ± 0.16	1.90 ± 0.19	1.79 ± 0.21		
Epinephrine (nmol/l)	Obese	0.21 ± 0.02	0.18 ± 0.02	0.18 ± 0.02	0.19 ± 0.02	NS	P<0.05
	Lean	0.28 ± 0.04	$0.31 \pm 0.05^*$	$0.30 \pm 0.04^*$	$0.30 \pm 0.04^*$		

Values are mean $\pm$ SEM for 11 obese and 13 lean subjects. Unpaired t-test: obese vs lean: * P<0.05, *** P<0.001.

Figure 6.2 Energy expenditure adjusted for fat free mass and lipid and carbohydrate oxidation rates before and during the infusion of a lipid heparin mixture in 11 obese (●) and 13 lean (Δ) men. Values are mean $\pm$ SEM. One-way repeated measurements ANOVA: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Baseline energy expenditure was significantly higher in the obese (table 6.2). After adjustment for FFM, baseline energy expenditure was similar in normal weight and overweight men (figure 6.2). Energy expenditure significantly increased during lipid heparin infusion. The increases were similar in both groups (obese vs lean: 0.34 ± 0.08 vs 0.40 ± 0.08 kJ/min, NS). RER was comparable between groups at baseline and decreased significantly during lipid heparin infusion (figure 6.2). Lipid oxidation was similar in both groups at baseline. During lipid heparin infusion, lipid oxidation increased to a similar extent in obese and lean men (Δ lipid oxidation: 19 ± 5 vs 13 ± 4 mg/min, NS) (table 6.2). Baseline carbohydrate oxidation did not differ between obese and lean men and significantly decreased during lipid heparin infusion. The decrease in carbohydrate oxidation was similar between groups (obese vs lean: -29 ± 12 vs -8 ± 6 mg/min, NS) (table 6.2). Expressed as percentage of total energy expenditure, lipid and carbohydrate oxidation were similar in both groups at baseline. During lipid heparin infusion, lipid oxidation increased and carbohydrate oxidation decreased similarly in both groups (figure 6.2).

Baseline plasma insulin levels were significantly higher in obese compared to lean men, but did not change during lipid heparin infusion in both groups (table 6.3). Plasma β -hydroxybutyrate concentrations were similar at baseline, but increased significantly more

($P < 0.05$) in the lean during lipid heparin infusion. Plasma norepinephrine and epinephrine concentrations did not differ significantly between groups at baseline. During lipid heparin infusion, plasma norepinephrine levels decreased significantly and plasma epinephrine levels were unchanged in both groups (table 6.3).

Discussion

The aim of the present study was to determine whether a certain increase in plasma NEFA levels leads to similar increases in lipid oxidation and energy expenditure in obese and lean men. Plasma NEFA levels were raised by infusing a lipid heparin mixture. Heparin was added to promote lipoprotein lipase activity and thus induce the hydrolysis of endogenous and exogenous triglycerides to NEFA and glycerol. The increases in plasma NEFA concentrations were similar in obese and lean men. Furthermore, the increases in lipid oxidation and thermogenesis were comparable between groups. Therefore, these data suggest that obese and lean men similarly increase their lipid oxidation and thermogenesis rates in response to a certain increase in plasma NEFA levels.

The increase in energy expenditure during lipid heparin infusion in our study was comparable with that found by Thiebaud *et al.*²³ in lean men. Jung *et al.*²⁴ examined both obese and lean subjects, which received an iv bolus of heparin with or without concomitant lipid infusion, but no two subjects received the same amount of lipids. No difference was found in the response between obese and lean subjects plotting the increase in NEFA level against the increase in energy expenditure, which is in accordance with our data. In contrast, Kjekshus *et al.*²⁵ found no changes in O_2 consumption and thus in thermogenesis during lipid heparin infusion. However, this could be due to methodological problems, since O_2 consumption was calculated from CO_2 output and volumetric changes produced by respiration. Our increase in lipid oxidation after a certain increase in plasma NEFA levels was comparable with the findings of Kleiber *et al.*²⁶ in lean subjects and Golay *et al.*²⁷ in obese subjects.

In order to exclude the possibility that increased SNS activity rather than increased NEFA concentration induced the increases in energy expenditure and lipid oxidation, plasma norepinephrine and epinephrine levels were measured. Plasma epinephrine levels did not change and plasma norepinephrine levels even slightly decreased during lipid heparin infusion in both groups. This is in accordance with the findings of Jung *et al.*²⁴, who reached much higher plasma NEFA concentrations in his experiment. This suggests that no additional SNS stimulation occurred during these studies. Plasma insulin levels, which inhibit NEFA release from adipose tissue, changed neither in obese nor in lean men during lipid heparin infusion, which is in accordance with the findings of Thiebaud *et al.*²³ The increase in energy expenditure and lipid oxidation is therefore likely to be induced directly by the increased NEFA availability. Furthermore, a recent study from our laboratory showed that inhibition of lipolysis, and thus a lower plasma NEFA availability, was accompanied by smaller increases in energy expenditure and lipid oxidation during β_1 -adrenoceptor stimulation.²⁸ This suggests that NEFA availability may be a limiting factor for increasing lipid oxidation and energy expenditure.

An increase in plasma NEFA concentration does not only lead to increases in lipid oxidation and thermogenesis, but also to an increase in ketone body production in the liver. Furthermore, elevated insulin concentrations seem to restrain NEFA-induced ketogenesis.²⁹ This is in line with our findings, showing that elevated plasma insulin concentrations in the obese were associated with a smaller increase in plasma β -hydroxybutyrate concentrations during lipid heparin infusion. Using the data of Keller *et al.*,²⁹ it can be calculated that total lipid oxidation rates might be overestimated by ~ 30%, if O_2 consumption is not corrected for the amount of O_2 needed for ketone body production. In our study, the increases in plasma β -hydroxybutyrate concentration were used to estimate the increases in ketone body production during lipid heparin infusion. It was found that the increase in lipid oxidation corrected for ketone body production did not significantly differ from that without correction in both groups. The larger increase in plasma β -hydroxybutyrate concentration in the lean therefore did not confound their increase in lipid oxidation, which remained comparable with that in the obese.

In this study, a certain raise in plasma NEFA level was accompanied by similar increases in lipid oxidation and thermogenesis in obese and lean men. Comparable results were found in a study in which we infused the selective β_1 -adrenoceptor agonist dobutamine. In this study, obese and lean men showed similar increases in plasma NEFA levels, lipid oxidation and energy expenditure in response to dobutamine.³⁰ Both studies suggest that obese subjects are capable of increasing their lipid oxidation and energy expenditure rates to the same extent as their lean counterparts. Furthermore, they provide no evidence for a difference in oxidative capacity in skeletal muscle between obese and lean men. The reduced increase in lipid oxidation in obese men during non-selective β -adrenergic stimulation, as found by Blaak *et al.*,¹⁰ or during selective β_2 -adrenergic stimulation, as found by Schiffelers *et al.*,³¹ might therefore be explained by a reduced increase in plasma NEFA concentration.

In conclusion, these data suggest that a certain increase in plasma NEFA concentration leads to similar increases in energy expenditure and lipid oxidation in obese and lean men. The accumulation of fat in obese subjects may therefore more likely be due to a defect in adipose tissue lipolysis than a defect in lipid oxidation.

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Nutrition Toxicology and Environment Research Institute Maastricht,
Departments of Human Biology¹ and Pulmonology²,
Maastricht University, Maastricht, The Netherlands.

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Abstract

Aim: To investigate whether development or maintenance of a relatively increased fat mass in normal weight patients with chronic obstructive pulmonary disease (COPD), despite periods of weight loss, may be related to an impaired β -adrenoceptor-mediated response in lipid utilization and thermogenesis.

Subjects: Nine male patients with COPD and 9 healthy male controls with a mean body mass index of 23.0 kg/m² (range: 15.6-29.2 kg/m²) and 23.8 kg/m² (range: 21.6-27.5 kg/m²), a mean fat mass of 19.0 kg (range: 6.4-30.2 kg) and 11.9 kg (range: 6.3-19.9 kg) and a mean age of 55 y (49-62 y) and 51 y (range: 41-58 y), respectively.

Design: The study protocol consisted of a 30 min baseline period, after which each subject received consecutive 30 min infusions of 6, 12 and 24 ng/kgFFM.min isoprenaline.

Measurements: Energy expenditure and respiratory exchange ratio (RER) were continuously measured by indirect calorimetry. At the end of each infusion period, a blood sample for the determination of plasma non-esterified fatty acids (NEFA), glucose, insulin, isoprenaline, norepinephrine and epinephrine concentration was taken.

Results: During β -adrenergic stimulation, NEFA levels increased significantly less in patients with COPD ($P < 0.001$). RER decreased similarly in both groups, indicating a similar change in the rate of lipid compared to carbohydrate oxidation during β -adrenergic stimulation. Energy expenditure increased similarly in both groups during β -adrenergic stimulation. However, since plasma isoprenaline concentrations were significantly higher in patients with COPD, thermogenesis related to plasma isoprenaline concentration was significantly reduced in this group ($P < 0.05$).

Conclusion: β -Adrenoceptor-mediated lipolysis and thermogenesis are impaired in normal weight COPD patients. This may play a role in the development or maintenance of their relatively increased fat mass, despite periods of weight loss.

Introduction

Weight loss commonly occurs in patients with chronic obstructive pulmonary disease (COPD), in particular in the emphysematous subtype.¹ Different patterns of body compositional changes are observed in these patients. Although normal weight loss merely comprises loss of fat and fat free mass, COPD patients may show a depletion of fat free mass despite a relative preservation of fat mass.^{2,3} In the latter group, functional capacity characterized by decreased muscle function, exercise capacity and even health status is more impaired as compared to underweight subjects with a normal fat free mass.⁴ Furthermore, recent studies indicate that the relative or absolute increase in fat mass and decrease in fat free mass in COPD patients might be related to intrinsic deviations in substrate metabolism, such as an impaired lipolytic response during exercise or insulin infusion⁵⁻⁷ and an increased fasting protein turnover.⁸

A blunted β -adrenergic response might also play a role in the development or maintenance of an increased fat mass.⁹ During the infusion of the non-selective β -adrenoceptor agonist isoprenaline, obese men showed an impaired response in lipolysis and lipid oxidation as compared to lean men,¹⁰ which favors the development or maintenance of their increased fat mass. Furthermore, when very obese men were compared with very lean men, an impaired thermogenic response was found as well.¹¹ After a weight loss period, these parameters remained impaired,⁹ suggesting that a diminished capacity to utilize fat may rather be a primary factor leading to the development of obesity rather than a secondary factor as a result of the obese state.

The relatively increased fat mass in normal weight patients with COPD might also be explained by a primary impaired response to β -adrenergic stimulation, but may also be secondary to their disease or its treatment. COPD patients with emphysema have increased plasma norepinephrine levels at rest,¹² suggesting an overstimulation of the sympathetic nervous system (SNS) in the basal state, whereas their chronic β_2 -adrenoceptor agonist use for bronchodilation causes downregulation of SNS responsiveness.^{13,14} Furthermore, several studies showed a decreased oxidative capacity in peripheral skeletal muscle which could blunt lipid utilization and thermogenesis.^{15,16}

The aim of the present study was to investigate whether development or maintenance of a relatively increased fat mass in normal weight patients with COPD, despite periods of weight loss, is related to an impaired response in lipid utilization and thermogenesis induced by β -adrenergic stimulation with isoprenaline.

Subjects and methods

Subjects

Nine male COPD patients with moderate to severe emphysema and 9 healthy male age-matched control subjects participated in this study. COPD was diagnosed according to the criteria of the American Thoracic Society¹⁷ and macroscopic emphysema was diagnosed by high-resolution computed tomography. All patients were in clinically stable condition and were weight stable for at least 3 months. However, on average they lost 3-4 kg of body weight

Table 7.1 Physical characteristics of COPD patients and control subjects.

Parameter	COPD		Control	
Body weight (kg)	71.7	(39.0-88.9)	73.5	(66.5-85.9)
Height (m)	1.76	(1.58-1.93)	1.76	(1.68-1.88)
Body mass index (kg/m ²)	23.0	(15.6-29.2)	23.8	(21.6-27.5)
Fat mass (kg)	19.0	(6.4-30.2)	** 11.9	(6.3-19.9)
Fat free mass (kg)	52.7	(32.6-66.5)	* 61.6	(53.6-71.8)
Age (y)	55	(49-62)	51	(41-58)
FEV ₁ (% of predicted)	36	(16-65)		
IVC (% of predicted)	84	(33-150)		

Values are mean (range) for 9 COPD patients and 9 healthy controls. FEV₁: forced expiratory volume in one second; IVC: inspiratory vital capacity. Unpaired t-test: COPD vs control: * $P < 0.05$, ** $P < 0.01$.

in the year preceding the experiment. Patients used inhaled β_2 -sympathomimetics and inhaled corticosteroids. In the 24 hours preceding the study, patients were not allowed to use any sympathomimetic drugs to prevent any acute effect of these drugs on energy metabolism. Control subjects were in good health as assessed by medical history and physical examination and none used β_2 -sympathomimetic drugs. Data on whole body thermogenesis of the control group have been published previously.¹⁸ Both patients and controls spent no more than 2 hours a week in organized sports activities. None of the subjects had a history of hypertension, cardiovascular disease or heart failure. Patients were all ex-smokers and controls were non-smokers. The study protocol was reviewed and approved by the Ethics Committee of Maastricht University and all subjects gave informed consent before participating in the study.

Experimental design

Subjects were studied in the morning after an overnight fast. They came to the laboratory by car or by bus to minimize the amount of physical activity before the test. On arrival, a canula was inserted into a forearm vein of each arm. One canula was used for the infusion of drugs and the other canula for the sampling of blood. All measurements were done with the subject in supine position and room temperature was kept at 21-23 °C. The study protocol consisted of four study periods. After a 30 min baseline measurement, subjects received consecutive infusions of 6, 12 and 24 ng/kg fat free mass (FFM).min isoprenaline (Isoprenaline sulfate, Fresenius, 's Hertogenbosch, The Netherlands), each dose for 30 min. At the end of each 30 min period, a blood sample was taken.

Clinical methods

Body composition of patients with COPD was measured by single frequency (50 kHz) bio-electrical impedance analysis (Xitron Technologies, San Diego, CA, USA) with the subject in supine position. FFM was calculated according to the equation of Schols *et al.*,¹⁹ validated for this group of patients. Body density of the control group was determined by hydrostatic

weighing with simultaneous lung volume measurement (Volugraph 2000, Mijnhardt, Bunnik, The Netherlands) and body composition was calculated according to the equation of Siri.²⁰

In patients with COPD, lung function was measured prior to the isoprenaline infusion test. Forced expiratory volume in one second (FEV₁) and inspiratory vital capacity (IVC) were calculated from the flow-volume curve using a spirometer (Jaeger, Hoechberg, Germany). Lung function was expressed as percentage of reference value.²¹

Whole body energy expenditure and respiratory exchange ratio (RER) were measured by an open circuit ventilated hood system (Oxycon beta, Mijnhardt, Bunnik, The Netherlands). The airflow rate and the O₂ and CO₂ concentrations of the in- and outflowing air were used to compute O₂ consumption and CO₂ production on-line through an automatic acquisition system connected to a personal computer. Energy expenditure was calculated according to the formula proposed by Weir.²² Energy expenditure and RER values were averaged over the last 10 min of each 30 min period during which steady state occurred.

Heart rate was monitored continuously by conventional electrocardiography and the mean value over the last 10 min of each 30 min period was used for further analysis.

Analytical methods

Blood samples for the determination of non-esterified fatty acids (NEFA), glucose and insulin were preserved in sodium-EDTA and those for isoprenaline, norepinephrine and epinephrine determination in heparin plus glutathione (1.5% wt/vol). Blood samples were immediately centrifuged for 10 min at 800 x *g* at 4 °C. Plasma was transferred into microtest tubes, rapidly frozen in liquid nitrogen and stored at -70 °C until further analysis. Plasma NEFA concentration was measured with the NEFA C kit (99475409, WAKO, Neuss, Germany) and plasma glucose concentration was measured with a glucose kit (Unimate 5, 0736724, Roche Diagnostica, Basel, Switzerland), both on a Cobas-Fara centrifugal analyzer (Roche Diagnostica, Basel, Switzerland). Plasma insulin concentration was determined with a double antibody radio-immunoassay (Insulin RIA 100, Pharmacia, Uppsala, Sweden). Plasma isoprenaline, norepinephrine and epinephrine levels were determined by high performance liquid chromatography according to the method of Smedes *et al.*²³ Standard samples with known concentrations were included in each run for quality control.

Data analysis

All data are presented as mean $\pm$ standard error of the mean (SEM). Data for energy expenditure were adjusted for FFM for group comparison using linear regression analysis.²⁴

To summarize the response of each subject to isoprenaline infusion in a single value, β -adrenergically-mediated thermogenesis was expressed as the dose and the plasma concentration of isoprenaline required to increase baseline energy expenditure by 15% (dose _{Δ EE=15%} and conc _{Δ EE=15%} respectively).¹¹ To evaluate the β -adrenergically-mediated heart rate response, both the chronotropic dose (CD25) and the chronotropic concentration (CC25) were calculated from the dose and plasma concentration of isoprenaline required to increase basal heart rate by 25 beats/min.²⁵ These values were determined by applying individual linear regression analysis to the measured response vs the dose or plasma concentration of isoprenaline.

Table 7.2 Respiratory exchange ratio (RER) and plasma glucose and insulin concentrations at baseline and during isoprenaline infusion in COPD patients and control subjects.

Parameter	Group	Isoprenaline (ng/kg FFM.min)				ANOVA	
		0	6	12	24	Parameter	Par x Group
RER	COPD	0.82 ± 0.01	0.83 ± 0.01	0.82 ± 0.01	0.81 ± 0.01	P<0.01	NS
	Control	0.82 ± 0.01	0.84 ± 0.01	0.81 ± 0.01	0.81 ± 0.01		
Glucose (mmol/l)	COPD	5.24 ± 0.21	5.16 ± 0.19	5.12 ± 0.19	5.33 ± 0.18	P<0.05	NS
	Control	5.00 ± 0.16	5.01 ± 0.12	5.03 ± 0.10	5.22 ± 0.08		
Insulin (mU/l)	COPD	5.09 ± 0.45	6.63 ± 0.56	7.26 ± 0.71	8.52 ± 0.96	P<0.001	NS
	Control	5.86 ± 0.97	6.51 ± 0.50	7.22 ± 0.81	9.30 ± 1.40		

Values are mean ± SEM for 9 COPD patients and 9 control subjects.

Figure 7.1 Plasma isoprenaline, norepinephrine and epinephrine concentrations during β-adrenergic stimulation with isoprenaline. Values are mean ± SEM for 9 COPD patients (●) and 9 healthy controls (Δ). One-way repeated measurements ANOVA: * $P < 0.05$, *** $P < 0.001$. Unpaired t-test: COPD vs control: * $P < 0.05$, *** $P < 0.001$.

The effect of β-adrenergic stimulation between groups was analyzed with two-way repeated measurements ANOVA. Post hoc testing was done with an unpaired t-test. The effect of β-adrenergic stimulation within a group was analyzed with one-way repeated measurements ANOVA. A P -value < 0.05 was regarded as statistically significant.

Results

Physical characteristics of the subjects are given in table 7.1. Body weight and body mass index were similar in the two groups. Although patients with COPD lost 3–4 kg body weight in the year preceding the experiment, they had a significantly higher fat mass ($P < 0.01$) and a significantly lower fat free mass ($P < 0.05$) as compared to control subjects.

Plasma isoprenaline concentrations significantly increased during isoprenaline infusion in COPD patients and control subjects, but were significantly higher in the patient group (figure 7.1). At baseline, plasma norepinephrine levels were significantly higher ($P < 0.05$) and plasma epinephrine levels were slightly lower ($P = 0.06$) in COPD patients as compared to controls. During β-adrenergic stimulation with isoprenaline, norepinephrine concentrations significantly increased and epinephrine concentrations significantly decreased in both groups. The changes in plasma norepinephrine and epinephrine concentration were comparable between groups (figure 7.1).

At baseline, plasma NEFA concentrations were similar in COPD patients and controls (figure 7.2). However, the increase in plasma NEFA concentration was significantly reduced

Figure 7.2 Plasma non-esterified fatty acids (NEFA) concentration, energy expenditure adjusted for fat free mass and heart rate expressed per dose isoprenaline infused. Values are mean $\pm$ SEM for 9 COPD patients (●) and 9 healthy controls (Δ). One-way repeated measurements ANOVA: ### $P < 0.001$. Unpaired t-test: COPD vs control: * $P < 0.05$, ** $P < 0.01$.

($P < 0.001$) in patients with COPD, despite the greater increase in isoprenaline concentration, suggesting a blunted β -adrenergically-mediated lipolytic response. Plasma glucose and insulin levels were similar in both groups at baseline. Glucose and insulin levels significantly increased during β -adrenergic stimulation, but these increases were not significantly different between groups (table 7.2).

Baseline energy expenditure was slightly lower in patients with COPD as compared to controls (4.71 ± 0.24 vs 5.21 ± 0.22 kJ/min, $P = 0.14$), but after adjustment for FFM, baseline energy expenditure was similar in both groups (figure 7.2). During β -adrenergic stimulation, energy expenditure significantly increased in both groups. There was no significant difference in the increase in energy expenditure between patients and control subjects. In addition, dose_{ΔEE=15%} was not different between groups (table 7.3). However, when responses were related to plasma isoprenaline concentrations, conc_{ΔEE=15%} was significantly higher ($P < 0.05$) in patients as compared to controls, indicating a blunted β -adrenergically-mediated thermogenic response in patients with COPD (figure 7.3, table 7.3). RER was similar in both groups at baseline and significantly decreased with isoprenaline, indicating a similar change in the rate of lipid to carbohydrate oxidation during β -adrenergic stimulation (table 7.2).

Heart rate was comparable in patients and controls at baseline and similarly increased during β -adrenergic stimulation (figure 7.2). Thus, CD25 was not significantly different between groups. When heart rate responses were related to plasma isoprenaline concentrations, CC25 was slightly higher in patients with COPD, but this difference did not reach statistical significance ($P = 0.11$) (figure 7.3, table 7.3).

Figure 7.3 Plasma non-esterified fatty acids (NEFA) concentration, energy expenditure adjusted for fat free mass and heart rate expressed per plasma isoprenaline concentration. Values are mean $\pm$ SEM for 9 COPD patients (●) and 9 healthy controls (Δ).

Discussion

The present study intended to investigate whether development or maintenance of a relatively increased fat mass in normal weight patients with COPD, despite periods of weight loss, is related to a blunted increase in lipid utilization and thermogenesis during β -adrenergic stimulation with isoprenaline. It was found that the β -adrenoceptor-mediated increase in NEFA concentration was impaired in COPD patients, indicating a blunted lipolytic response. RER decreased similarly in both groups, suggesting a similar change in the rate of lipid to carbohydrate oxidation. β -Adrenoceptor-mediated thermogenesis was impaired when it was related to plasma isoprenaline concentrations. The impaired release of NEFA from adipose tissue and the reduced thermogenic response may play a role in the development or maintenance of relatively increased fat stores in patients with COPD, even when losing weight.

COPD patients and control subjects showed a similar response in energy expenditure when related to the dose of isoprenaline infused. However, COPD patients had significantly higher plasma isoprenaline concentrations and thus an impaired thermogenic response when related to plasma isoprenaline concentration. The heart rate response was also slightly lower in patients with COPD when related to plasma isoprenaline concentration, but this did not reach statistical significance. The differences in plasma isoprenaline concentrations indicate that the pharmacokinetics of isoprenaline are different in patients as compared to healthy controls. The reason for this is not clear. Differences in hepatic or renal clearance are not evident, since none of the patients nor control subjects were diagnosed with impaired

Table 7.3 Energy expenditure adjusted for fat free mass and heart rate and their sensitivity to β -adrenergic stimulation in COPD patients and control subjects.

Parameter	COPD	Control
Baseline energy expenditure adjusted for FFM (kJ/min)	4.97 $\pm$ 0.13	4.95 $\pm$ 0.20
Dose _{ΔEE=15%} (ng/kg FFM.min)	27 $\pm$ 6	22 $\pm$ 5
Conc _{ΔEE=15%} (nmol/l)	1.80 $\pm$ 0.32	0.85 $\pm$ 0.23
Basal heart rate (beats/min)	65 $\pm$ 4	63 $\pm$ 4
CD25 (ng/kg FFM.min)	23 $\pm$ 5	24 $\pm$ 3
CC25 (nmol/l)	1.43 $\pm$ 0.25	0.93 $\pm$ 0.16

Values are mean $\pm$ SEM for 9 COPD patients and 9 control subjects. Dose _{Δ EE=15%} and conc _{Δ EE=15%}: dose or plasma concentration of isoprenaline needed to increase thermogenesis by 15%; CD25 and CC25: dose or plasma concentration of isoprenaline needed to increase heart rate by 25 beats/min. Unpaired t-test: COPD vs control: * $P < 0.05$.

liver or renal function. Another explanation might be a reduction in the number of β -adrenoceptor binding sites. Literature shows that a reduced β -adrenoceptor number on fat cells might be a primary factor leading to the development or maintenance of a relatively increased fat mass.²⁶ On the other hand, chronic β_2 -adrenoceptor agonist administration is also found to reduce the number of β -adrenoceptors on lymphocytes. Fourteen days of oral terbutaline administration is known to reduce the lymphocyte β -adrenoceptor number by more than 50% in both normal subjects and asthmatic patients.²⁷⁻²⁹ If the β -adrenoceptor number is reduced in these and other tissues and a similar dose of isoprenaline is given to patients with COPD and controls, less isoprenaline can bind to the available receptors in patients and as a consequence, the concentration free isoprenaline is increased in the patient group. Furthermore, the significantly higher plasma isoprenaline concentrations in patients with COPD make clear that individual plasma concentration-response curves instead of dose-response curves should be used in the analysis of these kind of experiments, since plasma concentration-response curves increase the precision of these infusion tests. This was already emphasized by others.^{11,18,25}

To our knowledge, this is the first study to report a blunted isoprenaline-induced increase in plasma NEFA concentration in COPD patients. However, the impaired NEFA release might be explained by a decreased lipolytic response and/or increased re-esterification within adipose tissue or other tissues. An impaired isoprenaline-induced lipolytic response has been reported before in obese subjects, both *in vivo*¹⁰ and *in vitro*,³⁰ indicating that this might be an important explanation for the blunted increase in NEFA concentration in COPD patients with a relatively increased fat mass.

This is also the first study to report an impaired thermogenic response in COPD patients with moderate to severe emphysema. Only Creutzberg *et al.*³¹ did a comparable study in which they measured the acute thermogenic effect after salbutamol nebulation. They found no difference in thermogenesis between patients with COPD and age-matched healthy control subjects. However, plasma salbutamol levels were not measured in this experiment, so a possibly reduced thermogenic response related to plasma salbutamol concentrations

could not be demonstrated. Furthermore, a reduced isoprenaline-induced increase in thermogenesis has been reported in obese subjects.¹¹ This blunted response might be explained by the impaired lipolytic response, which leads to a reduced NEFA availability in the blood. Therefore, less NEFA can be taken up and oxidized by skeletal muscle and as a consequence, thermogenesis may be reduced. This hypothesis is supported by another study from our group³² in which lipolysis was pharmacologically inhibited with acipimox. Concomitant β_1 -adrenergic stimulation resulted in a reduced increase in lipolysis and thermogenesis as compared to β_1 -adrenergic stimulation alone. Furthermore, reduced oxidative capacities in peripheral skeletal muscle, as reported both in obesity^{33,34} and COPD,^{15,16} might blunt lipid oxidation and thermogenesis.

The blunted β -adrenoceptor-mediated lipolytic and thermogenic response in patients with COPD might be a primary factor leading to the development of their relatively increased fat stores. This might be caused by an already developed impairment in β -adrenoceptor-mediated processes prior to the onset of the disease, as is the case in obesity. *In vitro* studies in fat cells from obese subjects suggest that the impaired lipolytic response to isoprenaline is related to a significant reduction in cell surface density of the β_2 -adrenoceptor.^{26,35} *In vivo*, we showed that β_2 -adrenoceptor-mediated thermogenesis and lipid utilization is blunted in obese compared to lean subjects, whereas β_1 -adrenoceptor-mediated responses are similar in both groups.³⁶

The blunted β -adrenergic response might also be secondary to the already developed increased fat stores. Since abdominal subcutaneous adipose tissue blood flow is reduced in obesity,³⁷ fat cell lipolysis might not be fully stimulated, leading to a reduced release of NEFA. Furthermore, due to a possibly reduced abdominal blood flow, as seen in obese subjects,³⁷ only part of the available NEFA in the interstitial fluid might be taken up into the blood stream and the remaining part has to be stored again. These factors might contribute to the maintenance of relatively increased adipose tissue stores.

Regression analysis showed that there was a significant relationship between the % body fat and the increase in plasma NEFA concentration ($r = -0.56$, $P < 0.02$) and the % body fat and the increase in plasma isoprenaline concentration ($r = 0.47$, $P < 0.05$) for the whole group. After reanalysis per subgroup (patients or controls), these significant relationships disappeared, probably due to the small number of subjects. Regression analysis between other combinations of variables, like increase in plasma NEFA concentration, increase in plasma isoprenaline concentration, baseline norepinephrine concentration and % body fat revealed no further significant relationships, not in the whole group nor in one of the subgroups. Whether the impaired β -adrenergic response in patients with COPD is a cause or a consequence of their increased fat mass needs to be further explored.

Another explanation for the blunted β -adrenergic response during isoprenaline infusion in patients with COPD might be desensitization of the SNS due to the disease. Patients with COPD are found to have increased plasma norepinephrine levels and decreased plasma epinephrine levels at rest.¹² This suggests that sympathetic nerve activity is increased in the basal state. Due to this chronic overstimulation of the SNS, β -adrenoceptors may become desensitized and consequently, the response to additional β -adrenergic stimulation might be blunted.

Finally, the impaired SNS response might be related to chronic usage of β_2 -adrenoceptor agonists for bronchodilation. In normal subjects, 2 weeks of regular salbutamol inhalation induced a blunted increase in plasma NEFA and glycerol concentrations during salbutamol infusion.³⁸ Thirteen days of salbutamol inhalation¹⁴ or 2 weeks of oral terbutaline administration¹³ induced impaired thermogenic responses after salbutamol inhalation or isoprenaline infusion, respectively. In COPD patients, the effect of chronic β_2 -adrenoceptor agonist usage on thermogenesis and lipid utilization has never been studied. However, in patients with moderate³⁹ to severe⁴⁰ asthma, which also use β_2 -adrenergic bronchodilators, a blunted increase in plasma NEFA concentration was found during epinephrine infusion. Furthermore, in asthmatic patients using high dosages of β -adrenergic bronchodilators, lymphocyte cAMP production during isoprenaline incubation was significantly reduced as compared to that in control subjects^{27,29,41} or asthmatic patients using non-adrenergic drugs.⁴¹ Moreover, when these asthmatic patients were changed to non-adrenergic drugs⁴¹ or placebo,²⁷ their cAMP response to isoprenaline returned to normal. Furthermore, Makino *et al.*⁴² reported that in asthmatic patients lymphocyte cAMP production was only impaired during incubation with salbutamol and not with norepinephrine ($\beta_1 > \beta_2$ -adrenoceptor affinity) as compared to healthy controls. This suggests that chronic β_2 -adrenoceptor agonist administration only desensitizes β_2 -adrenoceptors and not β_1 -adrenoceptors.

In our study, patients were asked to stop using their β_2 -adrenergic bronchodilators 24 h before the start of the experiment to prevent any acute interference with our isoprenaline infusion test. All patients used inhaled salbutamol (plasma $T_{1/2} = 4-6$ h) or inhaled salmeterol (plasma $T_{1/2}$ unknown due to very low plasma concentrations after inhalation) for bronchodilation. Considering 24 hours withdrawal, plasma salbutamol concentrations would be less than 5% of that directly after inhalation and therefore will not directly influence our study. However, the desensitizing effect of chronic β_2 -adrenoceptor agonist usage on the measured parameters is still present after 24 h withdrawal.

Our patients with COPD also chronically inhaled corticosteroids which are known to potentiate the effect of β -adrenergic stimulation. Hui *et al.*⁴³ showed that the reduction in lymphocyte β -adrenoceptor number after 3-5 weeks of oral terbutaline administration was completely reversed 16 h after a single intravenous dose of methylprednisolone in both normal subjects and asthmatic patients. Furthermore, Reynisdottir *et al.*³⁰ showed that lipolytic sensitivity to isoprenaline in isolated abdominal adipocytes from asthmatic patients (who only sporadically needed inhaled β_2 -adrenoceptor agonists) increased 50-fold after 7 days of oral prednisolone treatment. Sensitivity to terbutaline increased 25-fold, while that to dobutamine (β_1 -adrenoceptor agonist) remained unchanged after treatment. Furthermore, the number of β_2 -adrenoceptor binding sites increased by 60% after glucocorticosteroid treatment, whereas the number of β_1 -adrenoceptor binding sites was not affected. This suggests that glucocorticosteroids selectively increase β_2 -adrenoceptor density and function and may possibly reverse the desensitizing effect of chronic β_2 -adrenoceptor agonist usage.

In conclusion, β -adrenoceptor-mediated lipolysis and thermogenesis are reduced in normal weight patients with COPD with a relatively increased fat mass as compared to healthy age-matched control subjects. The impaired release of NEFA from adipose tissue and the reduced thermogenic response may play a role in the development or maintenance of the relatively increased fat stores in these patients, despite periods of weight loss.

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Stylidium giganteum is a member of the Stylidiaceae, a family of plants that is found in the tropics and subtropics of the Americas, Europe, and Africa. The family is named after the genus Stylidium, which is the most diverse and largest of the family. Stylidium giganteum is a perennial herb that grows to a height of 1-2 meters. It has a thick, fleshy, and somewhat flattened stem that is covered in small, dark, and scaly scales. The leaves are large, ovate, and have a prominent midrib. The flowers are large, tubular, and have a long, slender, and slightly curved corolla. The petals are large, ovate, and have a prominent midrib. The stamens are long, slender, and have a prominent anther. The pistil is long, slender, and has a prominent stigma. The fruit is a large, fleshy, and somewhat flattened capsule that is covered in small, dark, and scaly scales. Stylidium giganteum is a member of the Stylidiaceae, a family of plants that is found in the tropics and subtropics of the Americas, Europe, and Africa. The family is named after the genus Stylidium, which is the most diverse and largest of the family. Stylidium giganteum is a perennial herb that grows to a height of 1-2 meters. It has a thick, fleshy, and somewhat flattened stem that is covered in small, dark, and scaly scales. The leaves are large, ovate, and have a prominent midrib. The flowers are large, tubular, and have a long, slender, and slightly curved corolla. The petals are large, ovate, and have a prominent midrib. The stamens are long, slender, and have a prominent anther. The pistil is long, slender, and has a prominent stigma. The fruit is a large, fleshy, and somewhat flattened capsule that is covered in small, dark, and scaly scales.

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8 GENERAL DISCUSSION

General discussion

The research presented in this thesis focussed on the role of the different β -adrenoceptor subtypes of the SNS in the regulation of thermogenesis and lipid utilization in human obesity. In order to be able to draw valid conclusions on the role of the different β -adrenoceptor subtypes, we firstly examined which β -adrenoceptor agonists were specific for the β -adrenoceptor subtypes, we wanted to study. Secondly, we examined the mechanism behind the increase in lipid oxidation and thermogenesis during β_1 -adrenergic stimulation, since skeletal muscle, where these processes are presumed to be localized,¹ contains no β_1 -adrenoceptors but mainly β_2 -adrenoceptors.² Thirdly, since β -adrenoceptor-mediated thermogenesis³ and lipid utilization⁴ are impaired in the obese, we investigated whether the β_1 - and/or the β_2 -adrenoceptor or plasma non-esterified fatty acids (NEFA) availability is responsible for the impairment of these processes. Finally, we investigated whether the development or maintenance of a relatively increased fat mass in patients with chronic obstructive pulmonary disease (COPD), despite periods of weight loss, might be related to an impaired β -adrenergic response in thermogenesis and lipid utilization, as seen in obese subjects.^{4,5}

Specificity of β -adrenoceptor agonists

In order to study the *in vivo* role of the β_1 -, the β_2 - and the β_3 -adrenoceptor in human obesity, subtype-selective β -adrenoceptor agonists are needed. However, most available β -adrenoceptor agonists lose their specificity for a certain β -adrenoceptor subtype as the dose increases. Therefore, we studied the selectivity of dobutamine for the β_1 -adrenoceptor, of salbutamol for the β_2 -adrenoceptor and of isoprenaline in combination with nadolol or propranolol for the β_3 -adrenoceptor. If these β -adrenoceptor agonists at the dose tested were selective for the β -adrenoceptor subtype studied, we could use these agonists to study the role of the different β -adrenoceptor subtypes in thermogenesis and lipid utilization and its relation to human obesity.

β_1 - and β_2 -adrenoceptors

In our first study, described in chapter 2, we examined whether dobutamine and salbutamol can be used as selective β_1 - and β_2 -adrenoceptor agonists in *in vivo* studies on human thermogenesis and lipid utilization. Dobutamine in a dosage $\leq 10 \mu\text{g/kg.min}$ appeared to be specific for β_1 -adrenoceptors, since all dobutamine-induced effects on thermogenesis and lipid utilization were blocked by the selective β_1 -adrenoceptor antagonist atenolol (bolus: $42.5 \mu\text{g/kg}$, infusion: $1.02 \mu\text{g/kg.min}$) and thus no β_2 -adrenoceptor-mediated effects could be demonstrated.

A control test was done to evaluate the selectivity of the used dose of atenolol for β_1 - and β_2 -adrenoceptors. Therefore, β_2 -adrenoceptor-mediated effects of salbutamol (85 ng/kg.min) were compared with those during salbutamol plus atenolol infusion. We found that salbutamol-induced changes in energy expenditure and respiratory exchange ratio (RER) were not affected by atenolol. This suggests that atenolol did not block β_2 -adrenoceptor-mediated effects and therefore did not cover any β_2 -adrenoceptor-mediated effects of

dobutamine. Atenolol can therefore be used as a selective β_1 -adrenoceptor antagonist at the dosage given. However, after addition of atenolol, salbutamol-induced increases in plasma NEFA and glycerol concentration, heart rate and blood pressure were significantly reduced. This might be due to blockade of basal β_1 -adrenoceptor-mediated effects, but also blockade of β_1 -adrenoceptor-mediated effects of salbutamol cannot be excluded.

From these tests, we conclude that dobutamine at dosages $\leq 10 \mu\text{g/kg.min}$ can be used as a selective β_1 -adrenoceptor agonist in *in vivo* studies on human thermogenesis and lipid utilization. Salbutamol at a dosage of 85 ng/kg.min can be used as a selective β_2 -adrenoceptor agonist for the measurement of energy expenditure and lipid oxidation. However, if β_2 -adrenoceptor-mediated effects on plasma NEFA and glycerol concentrations are to be examined as indicators for lipolysis, salbutamol should be given in combination with atenolol, since additional β_1 -adrenoceptor-mediated effects of salbutamol on lipolysis cannot be excluded.

β_3 -adrenoceptors

In rats, β_3 -adrenergic stimulation leads to significant increases in thermogenesis and lipid utilization.^{6,7} However, the rat β_3 -adrenoceptor differs pharmacologically from the human β_3 -adrenoceptor^{8,9} and the specific β_3 -adrenoceptor agonists used in rats are only weak agonists in humans. At this moment, no selective β_3 -adrenoceptor agonist is registered for administration in humans. The non-selective β -adrenoceptor agonist isoprenaline might stimulate β_3 -adrenoceptors at an ~ 100 -fold higher dosage than the dosage at which it stimulates β_1 - and β_2 -adrenoceptors, as was shown in *in vitro* studies with isolated human fat cells.^{10,11} *In vivo* studies, however, show contradictory results on the ability of isoprenaline,¹² norepinephrine,⁴ and epinephrine⁴ to stimulate the human β_3 -adrenoceptor and therefore, it is still debated whether β_3 -adrenergic stimulation may lead to increases in human thermogenesis and lipid utilization. In chapter 3, we describe two studies in which an attempt was made to find evidence for a functional role of the β_3 -adrenoceptor in human thermogenesis and lipid utilization.

The first study was performed to investigate whether any significant increase in thermogenesis and/or lipid utilization during the infusion of $\sim 30 \text{ ng/kg.min}$ isoprenaline (β_1 -, β_2 - and β_3 -adrenoceptor agonist) would remain after full β_1 - and β_2 -adrenoceptor blockade. Isoprenaline-induced increases in heart rate and systolic blood pressure, as indicators for β_1 -adrenergic stimulation,¹³ could not be prevented by the administration of nadolol or propranolol in a range of 2.5 to 40 mg. An increase in isoprenaline-induced tremor score, as indicator for β_2 -adrenergic stimulation,¹³ could not be prevented by propranolol administration in a range of 2.5 to 7.5 mg. Overall, this suggests that no complete β_1 - and β_2 -adrenoceptor blockade was achieved after pretreatment with nadolol nor with propranolol at dosages $\leq 40 \text{ mg}$. The found increases in thermogenesis, lipid oxidation and lipolysis might therefore be explained by co-activation of β_1 - and/or β_2 -adrenoceptors. Therefore, this study provided no evidence for a functional role of the human β_3 -adrenoceptor in thermogenesis and lipid utilization.

In the second study, described in chapter 3, isoprenaline infusion rates were increased to 50, 100 and 200 ng/kg.min and 80 mg of nadolol was used for β_1 - and β_2 -adrenoceptor blockade. Isoprenaline infusion did not cause any significant changes in energy expenditure,

lipid oxidation and lipolysis as compared to saline infusion. Only a significant increase in heart rate occurred as compared to saline infusion, which might indicate that non-selective β -adrenergic stimulation by a high dosage of isoprenaline overcomes β_1 -adrenergic blockade. Thus, we conclude that after pretreatment with 80 mg of nadolol, isoprenaline infusion at a rate ≤ 200 ng/kg.min provided no evidence for a β_3 -adrenoceptor-mediated increase in thermogenesis and lipid utilization *in vivo* in humans.

In conclusion, to investigate the role of the different β -adrenoceptor subtypes in human thermogenesis, lipid oxidation and lipolysis, dobutamine at a dosage ≤ 10 μ g/kg.min can be used as selective β_1 -adrenoceptor agonist and salbutamol (85 ng/kg.min) in combination with atenolol (bolus: 42.5 μ g/kg, infusion: 1.02 μ g/kg.min) can be used as selective β_2 -adrenoceptor agonist. Furthermore, no evidence for a functional role of the human β_3 -adrenoceptor in thermogenesis and lipid utilization could be provided by using the non-selective β -adrenoceptor agonist isoprenaline without simultaneous β_1 - and β_2 -adrenergic stimulation.

NEFA availability

During β_1 -adrenergic stimulation, significant increases in energy expenditure and lipid oxidation are found.^{14,15} These increases are assumed to be localized predominantly in skeletal muscle,^{1,4} but this tissue contains mainly β_2 -adrenoceptors and presumably no β_1 -adrenoceptors.² β_1 -Adrenergic stimulation is therefore not likely to increase energy expenditure and lipid oxidation by direct stimulation of skeletal muscle. We propose that the elevated levels of NEFA in blood, caused by enhanced lipolysis due to stimulation of β_1 -adrenoceptors on fat cells, indirectly induce the rise in lipid oxidation and energy expenditure. This hypothesis was confirmed by the study described in chapter 4, which showed that during simultaneous inhibition of lipolysis with acipimox, β_1 -adrenergic stimulation with dobutamine resulted in a reduced increase in lipolysis and consequently in a reduced increase in lipid oxidation and thermogenesis. This suggests that part of the dobutamine-induced rise 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 directly be stimulated by dobutamine.

These findings rise the interesting question whether an increased plasma NEFA level without β_1 -adrenergic stimulation can increase energy expenditure and lipid oxidation as well. In chapter 6, we describe a study in which subjects received an infusion containing a lipid heparin mixture to rise plasma NEFA levels. In response to this mixture, energy expenditure and lipid oxidation significantly rose. Similar results were found by others.¹⁶⁻¹⁹ Furthermore, no changes in plasma epinephrine concentration and even a slight decrease in plasma norepinephrine concentration were found by us as well as by others,¹⁷ suggesting that no additional stimulation of the sympathetic nervous system (SNS) occurred during these tests. We therefore conclude that an increase in plasma NEFA concentration can induce energy expenditure and lipid oxidation without concomitant SNS stimulation.

Obesity

Several studies have shown that the increases in energy expenditure, lipid oxidation and lipolysis are impaired in the obese during norepinephrine,^{20,21} epinephrine^{22,23} or isoprenaline^{4,5} infusion. Until now, it is unclear which β -adrenoceptor subtype is responsible for the impaired responses in thermogenesis and lipid utilization.

β_2 -adrenoceptors

In the study presented in chapter 5, we examined the role of the β_1 - and the β_2 -adrenoceptor in the impaired responses in thermogenesis and lipid utilization in obesity. Therefore, a group of overweight and normal weight men received an infusion with dobutamine or salbutamol plus atenolol in the dosages determined in an earlier study (chapter 2). It was shown that β_1 -adrenoceptor-mediated processes rose similarly in obese and lean men. However, β_2 -adrenoceptor-mediated increases in thermogenesis, lipid oxidation and lipolysis were impaired in the obese. This suggests that obese subjects may have a defect in the β_2 -adrenoceptor itself, its density or the pathway it mediates.

At cellular level, catecholamines first bind to a β -adrenoceptor in the cell membrane, which is coupled to a G_s -protein and activates the membrane-bound enzyme adenylyl cyclase (figure 8.1). This enzyme enhances the breakdown of intracellular ATP to cAMP which activates protein kinase A, which in turn phosphorylates hormone-sensitive lipase. This enzyme becomes activated and catalyzes the breakdown of triglycerides. During this process of lipolysis, fatty acids are liberated and can leave the fat cells to be bound to albumin and transported in the blood stream for utilization in other organs. *In vitro* studies have shown that in subcutaneous abdominal fat cells, glycerol release is similar after

Figure 8.1 β -Adrenoceptor-mediated pathway for lipolysis.

incubation with dobutamine. However, in fat cells from obese subjects²⁴ or subjects with a low isoprenaline sensitivity,²⁵ glycerol release was reduced after incubation with isoprenaline or terbutaline (β_2 -adrenoceptor agonist). This appears to be due to a significant reduction in cell surface density of β_2 -adrenoceptors. Furthermore, after addition of forskolin (stimulating adenylate cyclase) or cAMP (activating protein kinase A), glycerol release from subcutaneous abdominal fat cells was similar in subjects with a high and a low isoprenaline sensitivity.²⁵ This suggests that the cascade of processes mediated by adenylate cyclase is fully functional. The only process preceding this step is the coupling of the β_2 -adrenoceptor to the G_s -protein, which in addition to the reduced β_2 -adrenoceptor density might be impaired in the obese as well. The coupling of the β_1 -adrenoceptor to the G_s -protein is assumed to be normal in overweight subjects, since no reduced β_1 -adrenoceptor density on the cell surface and no reduced lipolytic response after dobutamine administration is found, both *in vitro*^{24,25} and *in vivo* (chapter 5). It is unknown whether β_2 -adrenoceptor density on skeletal muscle cells is reduced in obese subjects, which might provide an explanation for the reduced response in lipid oxidation and thermogenesis (chapter 5).

Recently, a polymorphism in the hormone-sensitive lipase gene was found to be associated with obesity,^{26,27} but until now, no studies have been published on the relation between this polymorphism and the lipolytic response of fat cells. Moreover, two polymorphisms in the β_2 -adrenoceptor, the Arg16Gly²⁸ and the Gln27Glu²⁸⁻³¹ polymorphism, were found to be associated with obesity. Furthermore, Large *et al.*²⁹ showed that in subcutaneous abdominal fat cells, the Arg16Gly polymorphism was associated with a 5-fold increased sensitivity for terbutaline (β_2 -adrenoceptor agonist), but not with obesity, whereas the Gln27Glu polymorphism was associated with obesity, but not with β_2 -adrenoceptor function. In contrast, until now no studies have been published on possible associations between polymorphisms in the β_2 -adrenoceptor and skeletal muscle function or between polymorphisms in the β_1 -adrenoceptor and obesity.

In conclusion, β_2 -adrenoceptor-mediated increases in thermogenesis and lipid utilization are impaired in the obese. A disfunctioning of the β_2 -adrenoceptor or its density may therefore play a role in the etiology or maintenance of a relatively increased fat mass and consequently obesity.

NEFA-availability

The impaired response in thermogenesis and lipid oxidation during non-selective β - or β_2 -adrenergic stimulation (chapter 5) in obese subjects might be explained by the reduced increase in lipolysis. Less NEFA are released from the adipose tissue into the blood, thus less NEFA can be taken up by skeletal muscle and consequently lipid oxidation and thermogenesis are impaired as well. Furthermore, Blaak *et al.*⁴ showed that there was no net uptake of NEFA in skeletal muscle during β -adrenergic stimulation. Moreover, others found that obese women have a decreased capacity to oxidize substrates and have increased glycolytic and anaerobic capacities, as measured by the activity of several key enzymes in skeletal muscle biopsies.^{32,33} This suggests that lipid oxidation is impaired in the obese independent from NEFA availability. During β_1 -adrenergic stimulation, no impaired increase in plasma NEFA concentration and no impaired responses in thermogenesis and lipid oxidation were found in the obese (chapter 5). This suggests that overweight subjects have no impaired

capacity to take up and oxidize NEFA in skeletal muscle. Further evidence for this was provided by the study described in chapter 6, in which obese and lean men received an infusion with a lipid heparin mixture to increase plasma NEFA concentrations. We found that a similar rise in plasma NEFA concentration resulted in similar increases in thermogenesis and lipid oxidation, thus providing evidence for an unimpaired response in lipid oxidation in obese subjects under these conditions.

The impaired release of NEFA from the adipose tissue into the blood may play a role in the development or maintenance of relatively increased fat stores. An enhanced fat mass may therefore be predictive for an impaired β -adrenergic response. This hypothesis appeared to be valid in normal weight COPD patients, which despite periods of weight loss show a relative preservation of fat mass despite a depletion of fat free mass.^{34,35} During β -adrenergic stimulation with isoprenaline, patients with COPD had a reduced lipolytic and thermogenic response as compared to healthy control subjects (chapter 7).

Furthermore, there seems to be a close relationship between the increase in plasma NEFA concentration and the increase in energy expenditure and lipid oxidation (figure 8.2). For a certain rise in plasma NEFA concentration, similar responses in energy expenditure and lipid oxidation are found in obese men, COPD patients (both black symbols) and lean healthy subjects (white symbols). Based on all these pieces of evidence, we hypothesize that lipolysis is the rate-limiting step in inducing thermogenesis and lipid oxidation during β -adrenergic stimulation.

Figure 8.2 Relationship between the increase in plasma non-esterified fatty acids (NEFA) levels and the increase in energy expenditure and lipid oxidation during each infusion period in which dobutamine ($\blacktriangle$), salbutamol plus atenolol ($\blacksquare$), a lipid heparin mixture ($\bullet$) or isoprenaline ($\blacktriangledown$) was given to subjects with a relatively high % body fat (obese men or COPD patients) (black symbols) or healthy lean subjects (white symbols).

Blood flow

The reduced increase in plasma NEFA concentration during non-selective β -⁴ (chapter 7) or β -₂-adrenergic stimulation (chapter 5) in subjects with a relatively high fat mass may be related to an impaired blood flow response in the abdominal subcutaneous adipose tissue. Several authors have shown that fasting subcutaneous abdominal blood flow is reduced in

the obese.³⁶⁻³⁸ Furthermore, the increase in adipose tissue blood flow in overweight subjects is impaired after an oral glucose load,³⁷ a mixed meal³⁸ and during β -adrenergic stimulation.³⁶ This suggests that the delivery of β -adrenoceptor agonists or fatty acid transport proteins may be diminished and/or the rate of NEFA re-esterification may be enhanced, thus contributing to the relatively enhanced fat stores in the obese.

The reduced increase in lipid oxidation during non-selective β -⁴ or β_2 -adrenergic stimulation (chapter 5) and the reduced increase in energy expenditure during non-selective β -⁵ (chapter 7) or β_2 -adrenergic stimulation (chapter 5) in subjects with a relatively high fat mass also may be related to an impaired blood flow response in skeletal muscle. Several authors show that although skeletal muscle blood flow is similar at rest in obese and lean subjects,^{4,39,40} it is impaired in the obese after an oral glucose load,⁴⁰ during a hyperinsulinaemic euglycaemic clamp³⁹ and during β -adrenergic stimulation.⁴ This suggests that also a reduced increase in skeletal muscle blood flow, leading to a reduced delivery of NEFA, contributes to the impaired response in lipid oxidation and thermogenesis in obese subjects during β -adrenergic stimulation.

According to our studies, the frequently found impaired responses in blood flow are not likely to have occurred during β_1 -adrenergic stimulation (chapter 5), since the increases in lipolysis, lipid oxidation and thermogenesis were similar in obese and lean men. Furthermore, the clear associations between the rise in plasma NEFA concentration and the rises in lipid oxidation and thermogenesis, as depicted in figure 8.2, do not support a major role for an impaired skeletal muscle blood flow in the etiology or maintenance of obesity. These data suggest that NEFA availability in blood is the rate limiting step for skeletal muscle lipid oxidation and thermogenesis. A role for the adipose tissue blood flow during β_2 -adrenergic stimulation cannot be excluded, especially since blood vessels contain β_2 -adrenoceptors. An impaired adipose tissue blood flow might cause an impaired NEFA release and consequently impaired responses in thermogenesis and lipid oxidation during β_2 -adrenergic stimulation.

Insulin

The reduced lipolytic response during β_2 -adrenergic stimulation may be caused by the higher increase in insulin concentration in the obese group (chapter 5), for insulin inhibits lipolysis. But since obese subjects are to some extent insulin resistant according to their high baseline insulin levels, the impact of this higher increase is difficult to interpret.

In vitro studies show that β_2 -adrenoceptor-mediated lipolysis in fat cells is impaired in the obese.²⁴ Since no insulin was added to the incubation media, this suggests that a higher plasma insulin concentration is certainly not the only factor explaining the reduced increase in lipolysis during β_2 -adrenergic stimulation. Two studies investigated the role of insulin in epinephrine-induced thermogenesis in lean subjects *in vivo*. One study showed that epinephrine increased energy expenditure independently from insulin concentrations,⁴¹ whereas the other study found an inhibitory effect of insulin.⁴² No studies on the role of insulin during SNS stimulation in the obese have yet been published.

The most reliable data on the role of insulin in substrate metabolism come from studies in which obese and lean subjects are divided into subgroups according to their insulin sensitivity. Recently, Camastra *et al.*⁴³ showed that inhibition of lipid oxidation by insulin

during a hyperinsulinaemic euglycaemic clamp depends on an interaction between obesity and insulin resistance, whereas the increase in glucose-induced thermogenesis only depends on insulin sensitivity and not on obesity. In contrast, Segal *et al.*⁴⁴ showed that the magnitude of the increase in glucose-induced thermogenesis during a hyperinsulinaemic euglycaemic clamp depends on obesity and not on insulin sensitivity. Furthermore, they found that the thermogenic effect of a mixed meal is independently associated with both insulin sensitivity and obesity.⁴⁴ These data make clear that the role of insulin with respect to thermogenesis and lipid utilization in obesity is still debated.

In the study presented in chapter 6, no change in plasma insulin concentration was found during the infusion of a lipid heparin mixture in obese and lean men, thus from this study no statement can be made on the effect of insulin on lipid oxidation and thermogenesis. During β_2 -adrenergic stimulation, plasma insulin concentrations increased significantly more (two-way repeated measurements ANOVA: insulin x group: $P < 0.05$) (Δ insulin during highest salbutamol dosage: obese vs lean: 6.0 ± 1.0 vs 3.5 ± 0.8 mU/l, NS), but energy expenditure, lipid oxidation and lipolysis increased significantly less in the obese (chapter 5). During β_1 -adrenergic stimulation, plasma insulin levels rose slightly, but not significantly more in the obese (Δ insulin during highest dobutamine dosage: obese vs lean: 8.7 ± 3.5 vs 4.4 ± 0.8 mU/l, NS), but the responses in thermogenesis, lipid oxidation and lipolysis were comparable between groups (chapter 5). Comparing the results from the β_1 - and the β_2 -adrenoceptor study, it is striking to see that the changes in plasma insulin levels were almost identical between studies, whereas thermogenesis and lipid utilization were only impaired in the β_2 -adrenoceptor study. Furthermore, during non-selective β -adrenergic stimulation, plasma insulin concentrations changed similarly in patients with COPD and controls (Δ insulin during highest isoprenaline dosage: patients vs controls: 3.4 ± 0.7 vs 3.4 ± 1.5 mU/l, NS), whereas the increases in lipolysis and thermogenesis were impaired in COPD patients (chapter 7). Overall, the studies presented in chapters 5-7 suggest that insulin plays no major role in inhibiting the responses in thermogenesis and lipid utilization during β -adrenergic stimulation in the subjects studied. However, our insulin responses were rather small compared to those during a hyperinsulinaemic euglycaemic clamp^{43,44} or after a mixed meal,⁴⁵ so an inhibitory effect of higher insulin levels on the increase in thermogenesis and lipid utilization in obesity cannot be excluded.

Overall, the studies described in this thesis suggest that a reduced NEFA availability in the blood, caused by an impaired release of NEFA from the adipose tissue, is responsible for the impaired responses in energy expenditure and lipid oxidation in obese men during non-selective β - or β_2 -adrenergic stimulation. Furthermore, the impaired lipolytic response may play a role in the development or maintenance of relatively increased fat stores. Literature suggests that the impaired response in lipolysis may be caused by a disfunctioning of the β_2 -adrenoceptor or a reduced β_2 -adrenoceptor density, resulting in impaired β_2 -adrenoceptor-mediated increases in adipose tissue blood flow and lipolysis. Skeletal muscle blood flow and plasma insulin levels are thought to play a minor role in the impaired response to β -adrenergic stimulation.

Recommendations for future research

During β_2 -adrenergic stimulation, thermogenesis and lipid utilization are impaired in the obese. This may be explained by a ~ 60% reduction in cell surface density of β_2 -adrenoceptors.^{24,25} It would be interesting to know whether the decrease in β_2 -adrenoceptor density is due to changes in synthesis, degradation or internalization of this adrenoceptor subtype. Reynisdottir *et al.*²⁴ found that β_2 -adrenoceptor mRNA levels were similar in fat cells from obese and lean subjects. On the other hand, Lönnqvist *et al.*²⁵ found that β_2 -adrenoceptor mRNA levels were significantly reduced in fat cells from subjects with a low sensitivity for isoprenaline compared to subjects with a high sensitivity. Low mRNA levels may result in a lowered rate of synthesis of the β_2 -adrenoceptor protein. However, changes in β -adrenoceptor mRNA and β -adrenoceptor protein levels do not always run in parallel.⁴⁶ Therefore, it is possible that a defect in the translation of β_2 -adrenoceptor mRNA to β_2 -adrenoceptor protein may also play a role in the observed lower β_2 -adrenoceptor sensitivity.

Furthermore, it would be interesting to know whether there is a relation between the known polymorphisms in the β_2 -adrenoceptor and the regulation of energy and substrate metabolism during β_2 -adrenergic stimulation. In subcutaneous abdominal fat cells, the Arg 16Gly polymorphism was associated with a 5-fold increased sensitivity for terbutaline (β_2 -adrenoceptor agonist), but no association with β_2 -adrenoceptor binding capacity or obesity was found.²⁹ In addition, the reduction in body weight after a 3 months combined low-calorie diet and exercise regimen was significantly higher in obese women with the Arg16Gly polymorphism, although food intake and exercise level were similar.⁴⁷ The Gln27Glu polymorphism was negatively associated with obesity in sedentary subjects, but not in physically active subjects. Physical activity thus might counterbalance the effect of the Gln27 variant of the β_2 -adrenoceptor to increase body weight, body fat and obesity.⁴⁸ However, Hellström *et al.*³¹ showed a positive association between obesity and the Glu27 variant in the β_2 -adrenoceptor in obese females, whereas in males there was a negative correlation between Glu27 and obesity. Therefore, studies on the effects of polymorphisms in the β_2 -adrenoceptor need to be stratified for sex.

Until now, the *in vivo* impaired responses in energy expenditure and lipid utilization during β_2 -adrenergic stimulation in the obese have only been studied on whole body level. The next step would be to examine the effect of β_2 -adrenergic stimulation on abdominal subcutaneous adipose tissue and skeletal muscle level *in vivo*. Fluxes of NEFA, glycerol, glucose, lactate, O_2 and CO_2 can be determined by measuring blood flow and arterial and venous concentrations of these substrates over these tissue compartments. An infusion with [U - ^{13}C]-palmitate may be added to split up NEFA fluxes into NEFA uptake and NEFA release. Skeletal muscle and whole body lipid oxidation might be further quantified by measuring [$^{13}CO_2$]-concentrations in blood and breath.

Conclusions

- To study the role of the different β -adrenoceptor subtypes in human thermogenesis, lipid oxidation and lipolysis, dobutamine at a dosage $\leq 10 \mu\text{g/kg.min}$ can be used as selective β_1 -adrenoceptor agonist and salbutamol (85 ng/kg.min) in combination with atenolol (bolus: $42.5 \mu\text{g/kg}$, infusion: $1.02 \mu\text{g/kg.min}$) can be used as selective β_2 -adrenoceptor agonist. However, no evidence could be found for a β_3 -adrenoceptor-mediated increase in energy expenditure and lipid utilization during isoprenaline infusion after pretreatment with nadolol or propranolol.
- During simultaneous inhibition of lipolysis with acipimox, β_1 -adrenergic stimulation with dobutamine resulted in a reduced increase in energy expenditure and lipid oxidation. This suggests that the dobutamine-induced increase in energy expenditure depends on NEFA availability.
- β_1 -Adrenoceptor-mediated increases in lipolysis, lipid oxidation and thermogenesis were similar in obese and lean men, but β_2 -adrenoceptor-mediated increases were impaired in the obese. Their blunted response in lipid oxidation and energy expenditure might be explained by the reduced increase in β_2 -adrenoceptor-mediated lipolysis and/or blood flow, since a certain increase in plasma NEFA concentration leads to similar increases in lipid oxidation and thermogenesis in obese and lean men.
- The impaired β -adrenoceptor-mediated responses in thermogenesis and lipid utilization in obese men and in normal weight patients with COPD with a relatively increased fat mass, despite periods of weight loss, may play a role in the development or maintenance of these relatively increased fat stores.

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Summary

The sympathetic nervous system (SNS) plays an important role in the regulation of energy and substrate metabolism. The response on SNS activity is impaired in obesity and may play a role in the development or maintenance of the obese state. The research presented in this thesis focussed on the role of the different β -adrenoceptor subtypes of the SNS in the regulation of energy expenditure, lipid oxidation and lipolysis in human obesity.

In order to draw valid conclusions, we firstly examined which β -adrenoceptor agonists were specific for the β -adrenoceptor subtypes we wanted to examine. In the study presented in chapter 2, we showed that dobutamine at a dosage $\leq 10 \mu\text{g/kg.min}$ can be used as selective β_1 -adrenoceptor agonist and salbutamol at a dosage $\leq 85 \text{ ng/kg.min}$ in combination with atenolol (bolus: $42.5 \mu\text{g/kg}$, infusion: $1.02 \mu\text{g/kg.min}$) can be used as selective β_2 -adrenoceptor agonist. A functional role for the human β_3 -adrenoceptor in thermogenesis and lipid utilization could not be found, as described in chapter 3.

Secondly, we examined the mechanism behind the increase in lipid oxidation and energy expenditure during β_1 -adrenergic stimulation, since skeletal muscle, where these processes are presumed to be localized, contains no β_1 -adrenoceptors but mainly β_2 -adrenoceptors. Therefore, β_1 -adrenergic stimulation is not likely to increase thermogenesis and lipid oxidation by direct stimulation of skeletal muscle. The study described in chapter 4 showed that during simultaneous inhibition of lipolysis with acipimox, β_1 -adrenergic stimulation with dobutamine resulted in a reduced increase in energy expenditure and lipid oxidation. This suggests that the dobutamine-induced increase in thermogenesis depends on the availability of plasma non-esterified fatty acids (NEFA).

Non-selective β -adrenoceptor-mediated thermogenesis, lipid oxidation and lipolysis are impaired in the obese. We investigated whether it is the β_1 - and/or the β_2 -adrenoceptor which is responsible for the impairment of these processes. In the study presented in chapter 5, we infused dobutamine or salbutamol in combination with atenolol to examine whether β_1 - and/or β_2 -adrenoceptor-mediated increases in thermogenesis and lipid utilization were similar in obese and lean men. It was found that β_1 -adrenoceptor-mediated processes increased similarly in both groups. However, β_2 -adrenoceptor-mediated increases in thermogenesis, lipid oxidation and lipolysis were impaired in the obese. Literature suggests that these impaired responses might be explained by a disfunctioning of the β_2 -adrenoceptor or its density on the cell membrane.

The reduced increase in energy expenditure and lipid oxidation during β_2 -adrenergic stimulation in obese subjects might be explained by the reduced increase in lipolysis. Less NEFA are released from the adipose tissue into the blood, thus less NEFA can be taken up by skeletal muscle and consequently, lipid oxidation and thermogenesis are impaired as well. In chapter 5, we found no impaired increase in plasma NEFA concentration and no impaired responses in thermogenesis and lipid oxidation during β_1 -adrenergic stimulation in the obese. This suggests that obese subjects are capable of extracting normal amounts of NEFA from the blood and oxidizing NEFA in skeletal muscle. Further evidence for this was provided by the study described in chapter 6, in which obese and lean men received

an infusion with a lipid heparin mixture to increase plasma NEFA concentrations. We found that a certain increase in plasma NEFA concentration resulted in similar increases in thermogenesis and lipid oxidation in overweight and normal weight men. Moreover, there appeared to be a clear relationship between the increase in plasma NEFA concentration and the increases in thermogenesis and lipid oxidation when the results from the studies described in chapters 5-7 were combined. This suggests that the reduced NEFA availability may be responsible for the impaired responses in thermogenesis and lipid oxidation during β_2 -adrenergic stimulation in obese subjects (chapter 5).

In chapter 7, we investigated whether the development or maintenance of a relatively increased fat mass in normal weight patients with COPD, despite periods of weight loss, might be related to an impaired β -adrenergic response in lipid utilization and thermogenesis, as seen in obese subjects. During non-selective β -adrenergic stimulation with isoprenaline, COPD patients had a reduced thermogenic and lipolytic response as compared to healthy control subjects. The impaired release of NEFA from the adipose tissue into the blood and the restoring of circulating NEFA which cannot be oxidized may play a role in the development or maintenance of relatively increased fat stores.

Overall, the studies described in this thesis suggest that a reduced NEFA availability in the blood, caused by an impaired release of NEFA from the adipose tissue, is responsible for the impaired responses in energy expenditure and lipid oxidation in obese men during non-selective β - or β_2 -adrenergic stimulation. Furthermore, the impaired lipolytic response may play a role in the development or maintenance of relatively increased fat stores. Literature suggests that the impaired response in lipolysis may be caused by a disfunctioning of the β_2 -adrenoceptor or its density on the cell membrane. Blood flow and plasma insulin levels are thought to play a minor role in the impaired response on SNS activity, since the increases in energy expenditure and lipid utilization were similar in obese and lean men during β_1 -adrenergic stimulation.

Samenvatting

Het sympathisch zenuwstelsel speelt een belangrijke rol bij de regulatie van het energie- en substraatgebruik. Bij mensen met overgewicht is de respons op sympathische activiteit verminderd. Dit speelt mogelijk een rol bij het ontstaan of het handhaven van een relatief grote vetmassa. In dit proefschrift wordt beschreven welke rol de verschillende β -adrenoceptor subtypes van het sympathisch zenuwstelsel spelen in de regulatie van de vetafbraak, de vetverbranding en het energiegebruik bij mannen met overgewicht.

Om betrouwbare conclusies te kunnen trekken, hebben we eerst onderzocht welke β -adrenoceptor agonisten specifiek waren voor de β -adrenoceptor subtypes, die we wilden bestuderen. In het onderzoek beschreven in hoofdstuk 2 toonden we aan dat dobutamine in een concentratie $\leq 10 \mu\text{g/kg.min}$ gebruikt kan worden als selectieve β_1 -adrenoceptor agonist en dat salbutamol in een concentratie $\leq 85 \text{ ng/kg.min}$ in combinatie met atenolol (bolus: $42.5 \mu\text{g/kg}$, infuus: $1.02 \mu\text{g/kg.min}$) gebruikt kan worden als selectieve β_2 -adrenoceptor agonist. De studie in hoofdstuk 3 kon geen aanwijzingen verschaffen voor een functionele rol van de β_3 -adrenoceptor in het energie- en substraatgebruik van de mens.

Vervolgens hebben we het mechanisme onderzocht, dat schuilt achter de toename in het energiegebruik en de vetverbranding tijdens β_1 -adrenerge stimulatie. Aanleiding hiervoor is het feit dat de skeletspier, waarin deze processen verondersteld worden plaats te vinden, geen β_1 -adrenoceptoren bevat, maar voornamelijk β_2 -adrenoceptoren. Daarom is het niet waarschijnlijk, dat tijdens β_1 -adrenerge stimulatie de skeletspier op directe wijze gestimuleerd wordt om haar energiegebruik en vetverbranding te verhogen. De studie in hoofdstuk 4 laat zien, dat tijdens gelijktijdige remming van de vetafbraak met acipimox, de toename in het energiegebruik en de vetverbranding tijdens de infusie van dobutamine verminderd is. Dit suggereert, dat de beschikbaarheid van vrije vetzuren in het bloed mede bepalend is voor de toename in het energiegebruik en de vetverbranding tijdens β_1 -adrenerge stimulatie.

De toename in energiegebruik, vetverbranding en vetafbraak tijdens niet-selectieve β -adrenerge stimulatie is verminderd bij mensen met overgewicht. Wij onderzochten of deze verminderde respons veroorzaakt wordt door het disfunctioneren van de β_1 -adrenoceptor en/of de β_2 -adrenoceptor. In de studie beschreven in hoofdstuk 5 gaven we een groep mannen met en zonder overgewicht een infuus met dobutamine of salbutamol in combinatie met atenolol en maten vervolgens hun toename in het energiegebruik, de vetverbranding en de vetafbraak. Tijdens β_1 -adrenerge stimulatie bleken de veranderingen in deze processen vergelijkbaar te zijn tussen beide groepen. Tijdens β_2 -adrenerge stimulatie was de toename in het energiegebruik, de vetverbranding en de vetafbraak significant lager bij de mannen met overgewicht. De literatuur suggereert dat deze verminderde respons veroorzaakt wordt door een disfunctie van de β_2 -adrenoceptor of diens dichtheid op de celmembranen.

De verminderde toename in energiegebruik en vetverbranding tijdens β_2 -adrenerge stimulatie bij mannen met overgewicht kan verklaard worden door de verminderde toename in de vetafbraak. Hierdoor worden minder vrije vetzuren vanuit het vetweefsel afgegeven aan het bloed, waardoor de skeletspier minder vrije vetzuren uit het bloed kan opnemen en als gevolg daarvan de vetverbranding en het energiegebruik minder kan verhogen. Tijdens

β_1 -adrenerge stimulatie vertoonden de mannen met overgewicht geen verminderde toename in de concentratie vrije vetzuren in het bloed en geen verminderde respons in het energiegebruik en de vetverbranding. Dit suggereert dat mannen met overgewicht in staat zijn normale hoeveelheden vrije vetzuren uit het bloed op te nemen en vervolgens in de skeletspier te verbranden. Deze uitspraak wordt ondersteund door een andere studie (hoofdstuk 6), waarin een groep mannen met en zonder overgewicht een infuus met een vet/heparine-oplossing kregen. Uit deze studie bleek, dat een bepaalde toename in de hoeveelheid vrije vetzuren in het bloed leidde tot vergelijkbare toenames in energiegebruik en vetverbranding bij mannen met en zonder overgewicht. Wanneer de resultaten van de studies beschreven in hoofdstuk 5-7 gecombineerd werden, bleek er een duidelijke relatie te bestaan tussen de toename in de concentratie vrije vetzuren in het bloed en de toename in het energiegebruik en de vetverbranding. Dit suggereert dat de verminderde beschikbaarheid van vrije vetzuren in het bloed verantwoordelijk is voor de verminderde toename in het energiegebruik en de vetverbranding tijdens β_2 -adrenerge stimulatie bij mannen met overgewicht (hoofdstuk 5).

Als laatste onderzochten we of het ontstaan of handhaven van een relatief grote vetmassa, ondanks periodes van gewichtsverlies, bij patiënten met COPD gerelateerd is aan een verminderde toename in de vetafbraak en het energiegebruik tijdens β -adrenerge stimulatie (hoofdstuk 7), zoals eerder werd aangetoond bij mannen met overgewicht. Tijdens niet-selectieve β -adrenerge stimulatie met isoprenaline bleken COPD patiënten inderdaad een verminderde respons in de vetafbraak en het energiegebruik te vertonen in vergelijking tot de gezonde controles. De verminderde afgifte van vrije vetzuren uit het vetweefsel en het opnieuw opslaan van vrije vetzuren, die in de bloedbaan circuleren, maar niet in de skeletspier verbrand kunnen worden, speelt mogelijk een rol bij het ontstaan of handhaven van deze relatief grote vetvoorraden.

Samengevat suggereren de studies beschreven in dit proefschrift, dat de verminderde beschikbaarheid van vrije vetzuren in het bloed, veroorzaakt door een verminderde vetafbraak, verantwoordelijk is voor de lagere toename in energiegebruik en vetverbranding tijdens niet-selectieve β - of β_2 -adrenerge stimulatie. Daarnaast speelt de verminderde afgifte van vrije vetzuren uit het vetweefsel mogelijk een rol bij het ontstaan of het handhaven van een relatief grote vetvoorraad. In de literatuur wordt gesuggereerd, dat deze verminderde respons in de vetafbraak veroorzaakt wordt door het disfunctioneren van de β_2 -adrenoceptor of haar verminderde dichtheid op de celmembraan. De doorbloeding van het vet- en spierweefsel en de insuline concentratie in het bloed spelen waarschijnlijk een minder belangrijke rol in de verminderde respons op sympathische activiteit, aangezien de toename in energiegebruik, vetverbranding en vetafbraak tijdens β_1 -adrenerge stimulatie vergelijkbaar is tussen mannen met en zonder overgewicht.

Abbreviations

°C	degrees Celsius
AM	ante meridiem, before noon
ANOVA	analysis of variance
ATP	adenosine triphosphate
BMI	body mass index
BP	blood pressure
BW	body weight
cAMP	cyclic adenosine monophosphate
CHO cells	Chinese hamster ovary cells
CO ₂	carbon dioxide
COPD	chronic obstructive pulmonary disease
CV	coefficient of variation
HDL	high density lipoprotein
EC ₅₀	concentration required to produce a half-maximal response
EDTA	ethylene-diamine-tetra-acetate
EE	energy expenditure
FEV ₁	forced expiratory volume in one second
FFM	fat free mass
<i>g</i>	gravity
GC-MS	gas chromatography - mass spectrometry
GTP	guanosine triphosphate
G _s	stimulatory GTP-sensitive protein
h	hour
IU	international units
<i>iv</i>	intravenous
IVC	inspiratory vital capacity
kg	kilogram
K _i	equilibrium dissociation constant
kJ	kilojoule
l	liter
m	meter
µg	microgram
mg	milligram
min	minutes
MJ	megajoule
ml	milliliter
mmHg	millimeter mercury (hydrargyrum)
mU	milli-units
n	number
ng	nanogram

NEFA	non-esterified fatty acids
NS	not significant
O ₂	oxygen
P	probability
RER	respiratory exchange ratio
sec	seconds
SEM	standard error of the mean
SNS	sympathetic nervous system
vs	versus
y	year

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Iedereen BEDANKT!

Curriculum Vitae

Sandra Leonie Hendrika Schiffelers werd geboren op 27 oktober 1970 te Heerlen. In 1989 behaalde zij haar Gymnasium diploma aan het Bernardinuscollege te Heerlen. Aansluitend studeerde zij Voeding van de Mens aan de Landbouwuniversiteit te Wageningen. Tijdens haar studie deed zij een afstudeervak Klinische Voeding bij Prof WA van Staveren en Dr MA van Dusseldorp van de vakgroep Humane Voeding in samenwerking met het Kinder-oncologisch Centrum van het Radboudziekenhuis te Nijmegen. Vervolgens liep zij een half jaar stage bij Prof G Veereman-Wauters op de afdeling Kindergeneeskunde van de Universitaire Ziekenhuizen te Leuven (België). Als laatste deed zij een afstudeervak Fysiologie bij Dr VVAM Schreurs van de vakgroep Fysiologie van Mens en Dier. In augustus 1995 studeerde zij af in de richting Voeding en Gezondheid. Per 1 februari 1996 trad zij in dienst als Onderzoeker in Opleiding (OIO) bij de capaciteitsgroep Humane Biologie van de Universiteit Maastricht, alwaar het in dit proefschrift beschreven onderzoek werd uitgevoerd. Daarna werkte zij als Clinical Research Associate bij MedPass in Vaals. Sinds 1 oktober 2000 is ze als post-doc aangesteld bij de capaciteitsgroep Humane Biologie van de Universiteit Maastricht.

Publications

Full papers

- Schiffelers SLH, Brouwer EMC, van Baak MA, Saris WHM. Energie- en substraatgebruik tijdens β_1 -adrenerge stimulatie. *Voeding* 1997;56:33.
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