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Adrenergic- and non-adrenergically-mediated human adipose tissue lipolysis during acute exercise and exercise training

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ABSTRACT

Obesity-related adipose tissue dysfunction, in particular subcutaneous adipose tissue (SCAT) lipolysis, is characterized by catecholamine resistance and impaired ANP responsiveness. It remains unknown whether exercise training improves (non-)adrenergically-mediated lipolysis in metabolically compromised conditions. We investigated the effect of local combined α-/β-adrenoceptor blockade on abdominal SCAT lipolysis in lean insulin sensitive (IS, n=10), obese IS (n=10) and obese insulin resistant (IR, n=10) men. Obese men participated in a 12-week exercise training intervention to determine the effects on SCAT lipolysis. Abdominal SCAT extracellular glycerol concentration and blood flow (ATBF) were investigated using microdialysis, with/without local combined α-/β-adrenoceptor blockade at rest, during low-intensity endurance-type exercise and post-exercise recovery. In obese IR men, microdialysis was repeated after exercise intervention. The exercise-induced increase in SCAT extracellular glycerol was more pronounced in obese IS versus lean IS men, possibly resulting from lower ATBF in obese IS men. The exercise-induced increase in extracellular glycerol was blunted in obese IR versus obese IS men, despite comparable local ATBF. Abdominal SCAT extracellular glycerol was markedly reduced (remaining ~60% of exercise-induced SCAT extracellular glycerol) following local α-/β-adrenoceptor blockade in obese IS but not in IR men, suggesting reduced catecholamine-mediated lipolysis during exercise in obese IR men. Exercise training did not affect (non-)adrenergically-mediated lipolysis in obese IR men. Our findings show a major contribution of non-adrenergically-mediated lipolysis during exercise in male abdominal SCAT. Furthermore, catecholamine-mediated lipolysis may be blunted during exercise in obese IR men but could not be improved by exercise intervention, despite an improved metabolic profile and body composition.

Keywords: Exercise, Insulin resistance, Lipolysis, Natriuretic peptides, Obesity

Abbreviations list

ANP	atrial natriuretic peptide
AT	adipose tissue
ATBF	adipose tissue blood flow
AUC	area under the curve
FFM	fat free mass
iAUC	incremental area under the curve
IR	insulin resistant
IS	insulin sensitive
NP	natriuretic peptide
RM	repetition maximum
SCAT	subcutaneous adipose tissue
Wpeak	peak resistance

Introduction

Adipose tissue (AT) dysfunction is commonly observed in obesity and contributes to insulin resistance and chronic cardiometabolic diseases, including cardiovascular disease and type 2 diabetes (1). Disturbances in AT lipid metabolism, including decreased lipid uptake and impaired lipid mobilization are closely linked to ectopic fat deposition and obesity-related IR (2). An important function of the AT is to release fatty acids through lipolysis (3) during fasting and increased energy demanding conditions such as exercise. AT lipolysis is affected by multiple endocrine factors and impairments in its regulation have been identified in subcutaneous AT (SCAT) of obese humans (4). More specifically, β-adrenergically-mediated lipolysis is reduced (5) and inhibitory α_2-adrenoceptors become predominant on adipocytes in the obese insulin resistant state (6), leading to a blunted adrenergically-mediated lipolysis (5,7). Interestingly, local β-adrenergic blockade (alone or in combination with α_2-adrenergic blockade) in SCAT, inhibits exercise-induced lipolysis only to a minor extent at low-to-moderate intensities in healthy lean (8,9) and overweight individuals (10). Moro *et al.* (9) demonstrated that non-adrenergically-mediated lipolysis in SCAT substantially contributes to lipid mobilization during exercise in healthy young lean (9) and overweight men (10). Other key regulators of lipolysis are insulin and lactate, which both are able to inhibit AT lipolysis (11,12).

More recently, evidence showed that natriuretic peptides (NP) have pronounced effects in several key metabolic organs such as AT and skeletal muscle (13). Of interest, research indicated reduced circulating NP concentrations in human obesity and type 2 diabetes (14).

The latter findings, together with evidence that reduced systemic NP concentrations increase the risk of developing type 2 diabetes (15), highlight the importance of NP in metabolic disease. Of the NP family, atrial natriuretic peptide (ANP) is the most potent stimulator of human AT lipolysis (16). Interestingly, we recently demonstrated an impaired maximal ANP

responsiveness in isolated abdominal subcutaneous adipocytes of obese non-diabetic and type 2 diabetic men (17). In line, Rydén *et al.* (18) showed a blunted lipolytic effect of ANP in isolated abdominal subcutaneous adipocytes of obese women and *in situ* in abdominal SCAT of overweight men under resting conditions. Importantly, however, the physiological role of exercise-induced ANP-mediated lipolysis in obese individuals with different degrees of insulin sensitivity remains to be established.

Endurance exercise training has been known to partly improve β-adrenoceptor activity, reduce anti-lipolytic α_2-adrenoceptor sensitivity in human SCAT (19,20) and alleviate ANP-mediated lipolysis in subcutaneous adipocytes in young, metabolically healthy overweight individuals (21). However, to date, it remains elusive if combined endurance and resistance exercise training improves ANP-mediated lipolysis in insulin resistant obese individuals.

The aim of the present study was to examine the relative contribution of non-adrenergic regulation of abdominal SCAT extracellular glycerol concentration (marker of lipolysis) by applying local combined α-/β-adrenoceptor blockade using the microdialysis technique at rest, during an acute bout of low-intensity endurance-type exercise and during recovery from exercise in obese men with a different degree of insulin sensitivity (insulin sensitive (IS) versus insulin resistant (IR)) and age-matched lean IS men. Additionally, we investigated whether 12 weeks of combined endurance and resistance exercise training improved the metabolic profile and (non-)adrenergically-mediated abdominal SCAT lipolysis in obese IR men.

Subjects and Methods

Subjects

Ten middle-aged healthy lean IS, 10 obese IS and 10 obese IR men participated in the present study. Subjects were included when they had a stable body weight for at least 3 months prior to the intervention and had no contraindications for participation in an exercise training intervention. Major exclusion criteria were a history, or clinical symptoms, of heart, lung or kidney disease, presence of endocrine anomalies and/or the use of beta-blockers, glucose or lipid-lowering medication. Insulin sensitivity was assessed via homeostasis assessment of insulin resistance (HOMA-IR) (22). Subjects were classified as IS or IR when HOMA-IR was ≤ 2.3 (23) or ≥ 3.8 (24), respectively. Anthropometrics and blood pressure were determined and body composition was measured using a Dual Energy X-ray Absorptiometry scan (Hologic Series Delphi-A Fan Beam X-ray Bone Densitometer). One week before the investigational protocol, peak oxygen uptake (VO_{2peak}) was determined during a maximal cardiopulmonary exercise test performed on an electrical braked cycle ergometer (Gymna Ergofit Cycle 400, Bilzen, Belgium) by using an incremental procedure (work rate increased by 15W/min until volitional exhaustion). Heart rate (electrocardiography) was monitored continuously and VO_{2peak} was measured using a Metalyzer II (Cortex Medical, Leipzig, Germany). The study was approved by the Medical Ethical Committee of the Jessa Hospital and Hasselt University and performed in accordance with the declaration of Helsinki (2008). All individuals gave written informed consent prior to the start of the study.

Experimental protocol

After an overnight fast, a catheter was inserted into the antecubital vein for blood sampling. They consumed a standardized meal and snack the evening before the test (total energy: 626 kcal; 23.4g fat (10.4g saturated fat); 73.8g carbohydrates (of which 6.8g sugar); 28.8g

protein; 2.9g salt; 2.3g fibres) and abstained from exhausting activities 48 hours prior to the experimental protocol. Next, two microdialysis catheters (CMA 63, CMA Microdialysis AB, Stockholm, Sweden) were inserted percutaneously into the subjects' SCAT after epidermal anesthesia (EMLA® crème: lidocaine 2.5% and prilocaine 2.5%, AstraZeneca AB) at a distance of 6-8 cm from the umbilicus (one probe on the left and one probe on the right side). Probes were connected to a microinfusion pump (Harvard apparatus, Plato BV, Diemen, The Netherlands) and perfused with Ringer solution (in mmol/l: 147 sodium, 4 potassium, 2.25 calcium and 156 chloride; Fresenius Kabi BV, 's Hertogenbosch, The Netherlands). Ethanol (50 mmol/l) was added to the perfusate and determined both in the ingoing (perfusate) and outgoing (dialysate) fluid, using the ethanol outflow/inflow ratio, to semi-qualitatively estimate changes in local adipose tissue blood flow (ATBF). A higher ethanol out/in ratio, corresponding to a lower ethanol wash-out, reflects a lower regional ATBF. One microdialysis catheter was perfused with Ringer solution supplemented with ethanol (control), while the contralateral catheter was perfused with Ringer, supplemented with ethanol, 100 µmol/l phentolamine ($\alpha_{1,2}$-adrenergic receptor antagonist) (Regitin 10 mg/ml; Novartis Pharma BV, The Netherlands) and 100 µmol/l propranolol (nonselective β-adrenergic receptor antagonist) (Dociton 1 mg/ml, Mibe GmbH, Germany), concentrations that have previously been shown to completely block the adrenergically-mediated regulation of abdominal SCAT lipolysis in lean and obese individuals (25-27). After a 60-min equilibration period (recovery from insertion), two 30-min fraction of dialysate were collected at a flow rate of 0.3 µl/min for the calculation of probe recovery (28), after which the perfusion rate was increased to 2.0 µl/min for the remaining of the experiment. During rest (2.0 µl/min), three 15-min fractions of dialysate were collected from both sites to determine the extracellular glycerol concentration (reflecting basal lipolysis). Next, subjects performed a single bout of endurance exercise (60 min at a relative exercise intensity of 40%

VO_{2max}) on a cycle ergometer while heart rate was monitored continuously (Polar, Kempele, Finland). Exercise was followed by a 60-min recovery period in supine position. During exercise and recovery, dialysate samples were collected at 15-min intervals without disconnecting the probes from the microinfusion pumps. Dialysate samples (2.0 µl/min) were immediately stored at -80°C until analysis. Venous blood samples were taken at 15-min intervals throughout the experimental protocol, centrifuged at 4°C for 10 min at 1200*g* and plasma and serum was stored at -80°C until analysis.

Indirect calorimetry

Substrate oxidation rates (E%) and energy expenditure (kJ/min) were calculated from VO_2 and VCO_2 determined at rest and during submaximal exercise via indirect calorimetry using a Metalyzer II (Cortex Medical, Leipzig, Germany). Water intake was allowed *ad libitum* during exercise and recovery.

Exercise training protocol

Obese IS and IR subjects participated in a 12-week exercise training program (3 sessions per week) conform recent exercise training guidelines for obesity treatment (29) while being asked not to change their habitual diet. Each training session started with cycling (Excite Bike, Technogym, Zaventem, Belgium) for 45min at 65% VO_{2peak} (heart rate based), followed by resistance exercise of 5 large muscle groups at 65-70% of 1 RM. Training volume and load were gradually increased during the intervention. After the intervention, the experimental protocol was repeated by taking venous blood samples in the obese IS group and performing SCAT microdialysis in obese IR subjects, as described above. Post-intervention experimental protocol were performed at least 3 days after the finale exercise training session to exclude any potential acute training effects.

Biochemical analysis

Microdialysate samples were immediately stored at -80°C and were analysed for glycerol, glucose and lactate concentrations by means of bioluminescence on an ISCUS clinical microdialysis analyser (Mdialysis AB, Stockholm, Sweden). Ethanol concentrations in perfusate and dialysate were measured spectrophotometrically the same day using a Cobas Fara semi-automatic analyser (Roche Diagnostics, Basal, Switzerland) and using a standard ethanol assay kit (Boehringer Mannheim, Germany). Plasma free glycerol was measured after precipitation with an enzymatic assay (Enzytec^{TM} Glycerol, Roche Biopharm, Switzerland), automated on a Cobas Fara spectrophotometric autoanalyser (Roche Diagnostics, Basel, Switzerland). Plasma FFA, glucose and lactate were measured with enzymatic assays on an automated spectrophotometer (ABX Pentra 400 autoanalyser, Horiba ABX, Montpellier, France). Plasma ANP was measured using an enzyme immunoassay (RayBiotech, Norcross GA, USA). Serum insulin was determined with radioimmunoassay kits (Human Insulin specific RIA Kit, Millipore Corporation, MA, USA).Plasma adrenalin and noradrenalin were determined using high performance liquid chromatography with electrochemical detection (ClinRep® Complete Kit for Catecholamines in Plasma, RECIPE chemicals & Instruments GmbH, Munich, Germany).

Statistical analysis

All data are expressed as mean ± SEM. Subjects were excluded from analyses when microdialysate samples of 2 subsequent time points were missing, in order to maintain paired samples. Dialysate and systemic exercise responses were expressed as area under the curve (AUC) and incremental area under the curve (iAUC), calculated by the trapezoid method. Cross-sectional analyses (differences between groups and conditions) for microdialysis

extracellular glycerol data were analysed with two-way repeated-measures ANOVA. In case of significance, post-hoc analyses with Bonferroni correction were applied to identify significant within-group effects. Differences in plasma concentrations and substrate metabolism between groups were tested with one-way ANOVA and differences within groups were analysed by means of paired *t*-test. Intervention effects in the obese groups were analysed with two-way repeated-measures ANOVA (with pre- and post-intervention as conditions), with Bonferroni post-hoc correction to detect within-group effects. Three subjects dropped out of the exercise intervention due to medical (n=1) or motivational reasons (n=2) and were therefore excluded from the statistical analyses. SPSS 21 for Macintosh OS X was used for all calculations (IBM Corporation, Armonk, NY, USA). Level of statistical significance was set at $p<0.05$ (2-tailed), while $p<0.10$ was considered a tendency.

Results

Baseline

Clinical characteristics

Subjects' characteristics are presented in Table 1. By design, there were significant differences between the lean and the obese IS and/or obese IR group with respect to body weight, BMI, WH-ratio, whole-body fat percentage and fat mass (all $p<0.05$). Furthermore, HOMA-IR and fasting serum insulin were significantly higher in obese IR compared to the lean and obese IS men ($p<0.001$ for both parameters in both groups). Physical fitness ($VO_{2peak/FFM}$ and $W_{peak/FFM}$) was significantly lower in the obese IS and obese IR group as compared to the lean group ($p<0.01$ and $p<0.001$, respectively) (Table 1).

Systemic responses

Under resting conditions, plasma glycerol, FFA, glucose, lactate, ANP, adrenalin and noradrenalin were comparable between groups, while fasting serum insulin was higher in obese IR compared to lean and obese IS men (Figure 1A-H). During exercise, plasma glycerol, FFA, glucose, ANP, adrenalin and noradrenalin increased to the same extent in all groups (Figure 1). The exercise-induced increase in plasma lactate was most pronounced in obese IR as compared to lean men (p_{ANOVA}=0.044) (Figure 1D). The exercise-induced increase in plasma ANP was similar in all groups, but peak plasma ANP was reached earlier in the lean compared to the obese groups (p=0.034) (Figure 1E). Serum insulin levels were significantly higher during exercise in obese IR as compared to obese IS and lean IS men, with no differences between the latter two groups (Figure 1F). During recovery, plasma glycerol, glucose, adrenalin, noradrenalin and ANP decreased back to baseline concentrations (Figure 1), while plasma lactate tended to remain elevated in the obese IR group (p_{ANOVA}=0.061) (Figure 1D). Furthermore, plasma FFA remained significantly elevated in obese IR as compared to lean IS men (p_{ANOVA}=0.020) (Figure 1B). Serum insulin remained significantly elevated in the obese IR group compared to the obese IS and lean group (p_{ANOVA}<0.001, respectively), with no differences between the latter two groups (Figure 1F). Detailed systemic plasma responses during rest, exercise and recovery are shown in Supplemental Table A1.

Substrate oxidation and energy expenditure

Whole-body energy expenditure (kJ/min), RQ and substrate oxidation (E%) were comparable between groups at rest and during exercise (data not shown).

Microdialysis

Abdominal SCAT blood flow

At rest, lean men had a significantly lower ethanol out/in ratio, reflecting a higher adipose tissue blood flow (ATBF), compared to both obese groups ($p<0.01$), whilst no significant difference in ATBF was observed between both obese groups (Figure 2 A, C and E). Local α-/β-adrenergic blockade induced a significant increase in ethanol out/in ratio in the lean group (Figure 2A), indicative of a reduced ATBF, while this effect disappeared during exercise. Moreover, this effect was not observed in the obese IS or IR group (Figure 2 C and E). Exercise induced a decrease in ethanol out/in ratio, reflecting an increase in ATBF, in all groups. This exercise-induced increase in ATBF tended to be higher in lean as compared to obese IS and obese IR men ($p=0.093$, $p=0.087$, respectively), irrespective of local α-/β-adrenergic blockade (Figure 2 A, C and E). During recovery, ATBF returned to resting levels, with a significantly higher ATBF (*i.e.* a lower ethanol out/in ratio) in the lean group compared to both obese groups (Figure 2 A, C and E). Details with respect to ethanol out/in ratio during baseline, exercise and recovery are shown in Supplemental Table A2.

Abdominal SCAT lipolysis

In SCAT, resting extracellular glycerol was comparable between groups (Figure 2 B, D and F). Local α-/β-adrenergic blockade had no significant effects on resting extracellular glycerol in SCAT in either of the groups (Figure 2 B, D and F). During exercise, extracellular glycerol significantly increased in all groups. In obese IS men, exercise-induced increase in glycerol concentration ($AUC_{0\text{-}60}$) was higher compared to lean ($p=0.011$), but not obese IR men ($p=0.816$) (Figure 2 B, D and F). Local α-/β-adrenergic blockade induced a significant reduction in the exercise-induced increase in extracellular glycerol in the obese IS group ($p=0.020$), but not in the lean IS or obese IR group (Figure 2G). During recovery, extracellular glycerol decreased in all groups, but remained significantly elevated in the obese

IS as compared to the lean group, with no differences between both obese groups (Figure 2 B, D and F). Additionally, there were no significant effects of α-/β-adrenergic blockade on the extracellular glycerol during recovery in any of the groups (Figure 2 B, D and F). More details can be found in Supplemental Table A2.

Exercise training intervention

Clinical characteristics and systemic responses

In both obese groups, exercise training significantly reduced body weight, BMI, whole-body fat percentage as well as fat mass (Table 1). Whole-body insulin sensitivity (HOMA-IR) was significantly improved in the obese IR group (p_{time}=0.005), but not in the obese IS group. Furthermore, physical fitness ($VO_{2peak/FFM}$ and $W_{max/FFM}$) improved significantly following the 12-week exercise training intervention (Table 1). The training intervention significantly reduced resting plasma FFA (Figure 3 C-D) and tended to reduce fasting ANP in both obese groups (Figure 3 K-L). Resting plasma glucose increased in the IS group but not in the IR group (Figure 3 E-F). The training intervention did not significantly alter plasma glycerol (Figure 3 A-B), insulin (Figure 3 I-J), lactate (Figure 3 G-H), adrenalin (Figure 3 M-N) or noradrenalin (Figure 3 O-P). However, serum insulin at rest, during exercise and recovery remained elevated in the obese IR compared to obese IS group (Figure 3 I-J).

Exercise-induced increases in plasma glycerol (Figure 3 A-B), FFA (Figure 3 C-D), lactate (Figure 3 G-H) and adrenalin (Figure 3 M-N) were significantly blunted after the exercise training intervention. Peak ANP (Figure 3 K-L) tended to be reduced in both obese groups and the increase in plasma glycerol, insulin and glucose during exercise remained unchanged after intervention. In the recovery period, beside reduced plasma FFA (Figure 3 C-D) and adrenalin (Figure 3 M-N), no significant training-induced changes were observed. In addition, exercise training did not alter whole-body energy expenditure or substrate oxidation

(data not shown). Detailed post-intervention plasma responses are shown in Supplemental Table A3.

Abdominal SCAT blood flow and lipolysis

Following exercise intervention, SCAT extracellular glycerol release was investigated only in the obese IR group. Ethanol out/in ratio (Figure 4A) as well as extracellular glycerol during the experimental protocol (Figure 4B) were not altered after the intervention. Additionally, α-/β-adrenergic blockade had no significant effect on resting, exercise-induced or recovery-related extracellular glycerol concentrations in SCAT following exercise training intervention (Figure 4B; Figure 2G).

Discussion

The present study is the first to investigate (non-)adrenergically-mediated lipolysis during low-intensity endurance-type exercise in abdominal SCAT in obese IS and obese IR men as compared to lean IS men. Furthermore, we examined whether a 12-week exercise training intervention altered (non-)adrenergically-mediated SCAT lipolysis in obese IR men. Here, we demonstrated that the exercise-induced increase in abdominal SCAT extracellular glycerol concentration was more pronounced in obese IS as compared to lean IS men, which may at least partly be explained by the lower exercise-induced increase in ATBF in obese IS as compared to lean men. Interestingly, the exercise-induced increase in SCAT extracellular glycerol in obese IR men was blunted as compared to obese IS men, which suggests in view of the comparable local ATBF responses in both obese groups, a blunted exercise-induced lipolysis in the obese IR state. Exercise-induced increase in SCAT extracellular glycerol was substantially reduced (by ~40%) following local combined α-/β-adrenergic blockade in obese IS but not in obese IR men, which may be reflective of a reduced catecholamine-mediated

SCAT lipolysis during exercise in the obese IR group. Finally, exercise training intervention improved body composition, physical fitness and exercise-induced systemic responses in both obese groups, and insulin sensitivity in the obese IR group. However, this was not accompanied by changes in (non-)adrenergically-mediated extracellular glycerol in the SCAT of obese IR men. Collectively, our findings firstly indicate that exercise-induced SCAT lipolysis is predominantly mediated by non-adrenergic factors, most likely natriuretic peptides (NP) in middle-aged lean IS, obese IS and obese IR men and, secondly, that SCAT catecholamine-mediated lipolysis during exercise may be reduced in obese IR as compared to IS men.

We showed a more pronounced increase in exercise-mediated SCAT extracellular glycerol concentration in obese IS as compared to lean IS men. Although the lipolytic response in abdominal SCAT is often blunted in human obesity (5), higher exercise-induced extracellular glycerol concentrations in the obese IS group may likely be explained by the substantially lower local ATBF in the obese state (10,30), thereby leading to less diffusion of mobilized glycerol into the blood stream and accumulation of mobilized glycerol at the level of the SCAT. Local α-/β-adrenergic blockade reduced resting ATBF in lean but not in the obese men, which might suggest a reduced adrenergic sensitivity of ATBF in the obese state, as previously reported (26). Noteworthy, although ATBF was not assessed using the golden standard ^{133}Xe wash-out technique giving a more quantitative value for ATBF, the differences in ATBF using the ethanol dilution technique may be used to obtain an indication of relative changes and differences in local ATBF. In the present study, it is difficult to tease out effects on ATBF (response) and extracellular glycerol concentrations when comparing the lean and obese groups due to differential effects on local ATBF. Nevertheless, both obese groups did show similar patterns of local ATBF, which allows comparison of SCAT extracellular glycerol concentrations between these groups. Of interest, the differential effects

on SCAT extracellular glycerol between obese IS and IR men during exercise may be reflective of a reduced catecholamine-mediated lipolysis during exercise in the obese IR group. The latter finding is in line with previous studies showing that in obese IR individuals, the lipolytic activity of the β-adrenergic receptors is attenuated (5-7), while an increase in anti-lipolytic α_2-adrenoceptors in SCAT reduces exercise-mediated extracellular glycerol (26). Additionally, obese IR individuals often display lower plasma catecholamine responses to physical exercise (31), although the latter was not observed in our study. Therefore, the reduced exercise-mediated lipolytic response upon combined α-/β-adrenoceptor blockade in the obese IS men, as opposed to the obese IR group, might suggest differences in adrenergic receptor expression and sensitivity. The blunted lipolytic response in obese IR men might also partly be explained by the significantly higher fasting and exercise-induced insulin concentrations. Since the anti-lipolytic effects of insulin might be normal or only slightly impaired in obese adipose tissue (32-34), the observed hyperinsulinemia in the obese IR group might have contributed to the attenuated adrenergically-mediated SCAT lipolysis as compared to the obese IS group, as previously shown (35). Furthermore, increased plasma lactate may have contributed to the reduced lipolytic response in obese IR men, since lactate has been shown to inhibit lipolysis in human primary adipocytes *in vitro* (11).

The present study implies that non-adrenergic regulators of lipolysis play a major role during low-intensity endurance-type exercise. Previously, propranolol (25) and phentolamine (26,27) already showed to fully inhibit adrenergically-mediated SCAT lipolysis at concentrations as applied in the present study. The exercise-induced lipolytic response was only suppressed to a minor extent in all groups with a remaining SCAT extracellular glycerol of ~60% in the obese IS group, a portion that was even higher in the lean and obese IR group (Figure 2G). These results clearly indicate a major contribution of non-adrenergic regulators of SCAT lipolysis. Importantly, other (anti-)lipolytic factors, such as the parathyroid

hormone, cortisol and growth hormone are less important during the current applied type and duration of exercise (4,10). Noteworthy, ANP may be responsible for the exercise-induced increase in SCAT extracellular glycerol, especially since ANP is one of the major lipolytic hormones produced upon exercise (16,36), next to sympathetic nervous system activation. In line with our findings, it has previously been shown that non-adrenergically mechanisms are involved in SCAT lipolysis, accounting for ~65% of the exercise-mediated lipolysis in young healthy lean and overweight men (9,10), being more pronounced in young healthy overweight as compared to lean men (10). It therefore is suggested that ANP-mediated SCAT lipolysis is particularly important in the overweight and obese state, despite displaying a lower *in vivo* ANP responsiveness in SCAT of overweight (18) and obese (17,18) individuals. In contrast to obese IS men, we found that SCAT extracellular glycerol in obese IR men was not affected by local α-/β-adrenergic blockade, which may propose an interaction between SCAT adrenergically-mediated lipolysis and whole-body insulin resistance and thus supports a catecholamine-resistant phenotype of the SCAT during exercise in the obese IR state. Therefore, a major role for ANP in SCAT lipolysis during exercise can be suggested, which is sustained in the obese IR state.

The 12-week exercise intervention increased insulin sensitivity in the obese IR group. While plasma glycerol, FFA, lactate, ANP and adrenalin were significantly reduced after the intervention, only minor reductions were observed for circulating glucose, insulin and noradrenalin. However, local exercise-induced abdominal SCAT extracellular glycerol was not improved following the exercise intervention. Moreover, local α-/β-adrenergic blockade efficacy was not affected by exercise training, although (based on individual data) a slightly more pronounced decrease in SCAT extracellular glycerol under local α-/β-adrenergic blockade was observed in 5 out of 8 obese IR men following exercise intervention. Together, these data suggest that even after substantial improvements in metabolic profile and body

composition after a 12-week exercise intervention, the reduced catecholamine-mediated lipolysis during exercise remains unaffected in obese IR men.

Although evidence suggests that exercise training beneficially affects SCAT insulin sensitivity (20), SCAT adrenergic sensitivity (20,37,38) and ANP-mediated lipolysis (39), future mechanistic studies are needed to obtain a better understanding of the exercise-induced hormonal lipolytic regulation in lean and obese individuals with a different degree of insulin sensitivity. Unfortunately, a natriuretic peptide receptor type A agonist/antagonist for use in humans is currently unavailable, which hampers strong conclusions about the physiological role of ANP in human AT lipolysis *in vivo*.

In conclusion, this study demonstrated a major role for non-adrenergically-mediated lipolysis in SCAT of middle-aged lean and obese men during low-intensity exercise, likely involving ANP-mediated lipolysis. Furthermore, obese IR men exhibit a reduced catecholamine-mediated lipolysis during exercise, whilst exercise training intervention did not reverse this impairment, despite improvements in metabolic profile and body composition.

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Declarations of interest

The authors report no conflicts of interest in this work.

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Author contribution statement

KV, RS, IW and IF performed the experiments. KV and RS performed sample analyses, statistical analysis and wrote the manuscript. KV, RS, IW and IF revised the manuscript. DH, BOE, EEB, GHG, JWJ designed the study and revised the manuscript. KV, RS, DH, EEB, GHG and JWJ contributed to data interpretation and editing of the manuscript. All authors approved the final version of the manuscript.

Clinical perspectives

- The relative physiological contribution of adrenergically- and non-adrenergically-mediated lipolysis in adipose tissue during acute exercise and following exercise training in obese individuals remains to be established.
- The present study demonstrated a major role for non-adrenergically-mediated lipolysis during acute exercise in both lean and obese individuals, and a reduced adrenergically-mediated lipolysis in obese insulin resistant individuals. A 12-week exercise training program did not improve the lipolytic impairments in abdominal subcutaneous adipose tissue of obese insulin resistant men, despite improved metabolic profile and body composition.
- Optimized exercise strategies are warranted to improve the regulation of adipose tissue lipolysis, especially in obese insulin resistant individuals.

References

1. Goossens GH. The role of adipose tissue dysfunction in the pathogenesis of obesity-related insulin resistance. Physiol Behav. 2008; 94:206-218

2. Stinkens R, Goossens GH, Jocken JW, Blaak EE. Targeting fatty acid metabolism to improve glucose metabolism. Obes Rev. 2015; 16:715-757

3. Jocken JW, Blaak EE. Catecholamine-induced lipolysis in adipose tissue and skeletal muscle in obesity. Physiol Behav. 2008; 94:219-230

4. Hansen D, Meeusen R, Mullens A, Dendale P. Effect of acute endurance and resistance exercise on endocrine hormones directly related to lipolysis and skeletal muscle protein synthesis in adult individuals with obesity. Sports Med. 2012; 42:415-431

5. Jocken JW, Goossens GH, van Hees AM, Frayn KN, van Baak M, Stegen J, et al. Effect of beta-adrenergic stimulation on whole-body and abdominal subcutaneous adipose tissue lipolysis in lean and obese men. Diabetologia. 2008; 51:320-327

6. Reynisdottir S, Wahrenberg H, Carlstrom K, Rossner S, Arner P. Catecholamine resistance in fat cells of women with upper-body obesity due to decreased expression of beta 2-adrenoceptors. Diabetologia. 1994; 37:428-435

7. Lafontan M, Berlan M. Fat cell adrenergic receptors and the control of white and brown fat cell function. J Lipid Res. 1993; 34:1057-1091

8. Arner P, Kriegholm E, Engfeldt P, Bolinder J. Adrenergic regulation of lipolysis in situ at rest and during exercise. J Clin Invest. 1990; 85:893-898

9. Moro C, Polak J, Hejnova J, Klimcakova E, Crampes F, Stich V, et al. Atrial natriuretic peptide stimulates lipid mobilization during repeated bouts of endurance exercise. Am J Physiol Endocrinol Metab. 2006; 290:E864-E869

10. Moro C, Pillard F, de Glisenzinski I, Klimcakova E, Crampes F, Thalamas C, et al. Exercise-induced lipid mobilization in subcutaneous adipose tissue is mainly related to

natriuretic peptides in overweight men. Am J Physiol Endocrinol Metab. 2008; 295:E505-E513

11. Liu C, Wu J, Zhu J, Kuei C, Yu J, Shelton J, et al. Lactate inhibits lipolysis in fat cells through activation of an orphan G-protein-coupled receptor, GPR81. J Biol Chem. 2009; 284:2811-2822

12. Langin D. Adipose tissue lipolysis revisited (again!): lactate involvement in insulin antilipolytic action. Cell Metab. 2010; 11:242-243

13. Verboven K, Hansen D, Jocken JWE, Blaak EE. Natriuretic peptides in the control of lipid metabolism and insulin sensitivity. Obes Rev. 2017; 18:1243-1259

14. Khan AM, Cheng S, Magnusson M, Larson MG, Newton-Cheh C, McCabe EL, et al. Cardiac natriuretic peptides, obesity, and insulin resistance: evidence from two community-based studies. J Clin Endocrinol Metab. 2011; 96:3242-3249

15. Magnusson M, Jujic A, Hedblad B, Engrström G, Persson M, Struck J, et al. Low plasma level of atrial natriuretic peptide predicts development of diabetes: the prospective Malmo Diet and Cancer study. J Clin Endocrinol Metab. 2012; 97:638-645

16. Sengenes C, Berlan M, De Glisezinski I, Lafontan M, Galitzky J. Natriuretic peptides: a new lipolytic pathway in human adipocytes. FASEB J. 2000; 14:1345-1351

17. Verboven K, Hansen D, Moro C, Eijnde BO, Hoebers N, Knol J, et al. Attenuated atrial natriuretic peptide-mediated lipolysis in subcutaneous adipocytes of obese type 2 diabetic men. Clin Sci (Lond). 2016; 130:1105-1114

18. Ryden M, Bäckdahl J, Petrus P, Thorell A, Gao H, Coue M, et al. Impaired atrial natriuretic peptide-mediated lipolysis in obesity. Int J Obes (Lond). 2016; 40:714-720

19. Stich V, de Glisezinski I, Galitzky J, Hejnova J, Crampes F, Rivière D, Berlan M. Endurance training increases the beta-adrenergic lipolytic response in subcutaneous adipose tissue in obese subjects. Int J Obes Relat Metab Disord. 1999; 23:374-381

20. Polak J, Moro C, Klimcakova E, Hejnova J, Majercik M, Viguerie N, et al. Dynamic strength training improves insulin sensitivity and functional balance between adrenergic alpha 2A and beta pathways in subcutaneous adipose tissue of obese subjects. Diabetologia. 2005; 48:2631-2640

21. Moro C, Pillard F, de Glisezinski I, Harant I, Rivière D, Stich V, et al. Training Enhances ANP Lipid-Mobilizing Action in Adipose Tissue of Overweight Men. Medicine & Science in Sports & Exercise. 2005; 37:1126-1132

22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28(7):412-419

23. Blaak EE, Hul G, Verdich C, Stich V, Martinez A, Petersen M, et al. Fat oxidation before and after a high fat load in the obese insulin-resistant state. J Clin Endocrinol Metab. 2006; 91:1462-1469

24. Wildman RP, Muntner P, Reynolds K, McGinn AP, Rafpathak S, Wylie-Rosett J, Sowers MR. The obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering: prevalence and correlates of 2 phenotypes among the US population (NHANES 1999-2004). Arch Intern Med. 2008; 168:1617-1624

25. Moro C, Crampes F, Sengenes C, de Glisezinski I, Galitzky J, Thalamas C, et al. Atrial natriuretic peptide contributes to physiological control of lipid mobilization in humans. FASEB J. 2004; 18:908-910

26. Stich V, de Glisezinski I, Crampes F, Hejnova J, Cottet-Emard JM, Galitzky J, et al. Activation of alpha(2)-adrenergic receptors impairs exercise-induced lipolysis in SCAT of obese subjects. Am J Physiol Regul Integr Comp Physiol. 2000; 279:R499-504

27. Polak J, Moro C, Bessière D, Hejnova J, Marquèes MA, Bajzova M, et al. Acute exposure to long-chain fatty acids impairs {alpha}2-adrenergic receptor-mediated

antilipolysis in human adipose tissue. J Lipid Res. 2007; 48:2236-2246

28. Goossens G, Blaak E, Saris W, van Baak M. Angiotensin II-induced effects on adipose and skeletal muscle tissue blood flow and lipolysis in normal-weight and obese subjects. J Clin Endocrinol Metab. 2004; 89:2690-2696

29. Yumuk V, Tsigos C, Fried M, Schindler K, Busetto L, Micic D, et al. European Guidelines for obesity management in adults. Obes Facts. 2015; 8:402-424

30. Bulow J. Human adipose tissue blood flow during prolonged exercise, III. Effect of beta-adrenergic blockade, nicotinic acid and glucose infusion. Scand J Clin Lab Invest. 1981; 41:415-424

31. Zouhal H, Lemoine-Morel S, Mathieu ME, Casazza GA, Jabbour G. Catecholamines and obesity: effects of exercise and training. Sports Med. 2013; 43:591-600

32. Lafontan M, Langin D. Lipolysis and lipid mobilization in human adipose tissue. Prog Lipid Res. 2009; 48:275-297

33. Jocken JW, Goossens GH, Boon H, Mason RR, Essers Y, Havekes B, et al. Insulin-mediated suppression of lipolysis in adipose tissue and skeletal muscle of obese type 2 diabetic men and men with normal glucose tolerance. Diabetologia. 2013; 56:2255-2265

34. Hershkop K, Besor O, Santoro N, Pierpont B, Caprio S, Weiss R. Adipose Insulin Resistance in Obese Adolescents Across the Spectrum of Glucose Tolerance. J Clin Endocrinol Metab. 2016; 101:2423-2431

35. Zhang J, Hupfeld CJ, Taylor SS, Olefsky JM, Tsien RY. Insulin disrupts beta-adrenergic signalling to protein kinase A in adipocytes. Nature. 2005; 437:569-573

36. Follenius M, Brandenberger G. Increase in atrial natriuretic peptide in response to physical exercise. Eur J Appl Physiol Occup Physiol. 1988; 57:159-162

37. De Glisezinski I, Crampes F, Harant I, Berlan M, Hejnova J, Langin D, et al. Endurance training changes in lipolytic responsiveness of obese adipose tissue. Am J Physiol. 1998;

275:E951-E956

38. Richterova B, Stich V, Moro C, Polak J, Klimcakova E, Marjercik M, et al. Effect of endurance training on adrenergic control of lipolysis in adipose tissue of obese women. J Clin Endocrinol Metab. 2004; 89:1325-1331

39. Moro C, Pasarica M, Elkind-Hirsch K, Redman LM. Aerobic exercise training improves atrial natriuretic peptide and catecholamine-mediated lipolysis in obese women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2009; 94:2579-2586

Table 1 Participants' characteristics and training intervention effects

	Lean (n = 10)	Obese insulin sensitive (n = 10)	Obese insulin resistant (n = 10)	P_{ANOVA}
Age, years	45 ± 2	47 ± 2	43 ± 1	0,527
Body weight (kg)	79,9 ± 2,9	101,5 ± 3,2 **	109,6 ± 4,7 ***	**<0,001**
Body mass index (kg/m²)	23,7 ± 0,4	32,6 ± 0,4 ***	33,9 ± 0,7 ***	**<0,001**
Waist-to-hip ratio	1,00 ± 0,00	1,04 ± 0,01 *	1,05 ± 0,01 *	**0,010**
Fat mass (kg)	16,4 ± 1,1	30,0 ± 1,7 ***	34,2 ± 1,8 ***	**<0,001**
Fat percentage (%)	22,0 ± 0,8	31,4 ± 0,9 ***	33,2 ± 1,1 ***	**<0,001**
Fat free mass (kg)	57,4 ± 1,8	65,0 ± 1,5	68,4 ± 2,8 **	**0,004**
Plasma glucose (mmol/L)	5,5 ± 0,0	5,2 ± 0,1	5,8 ± 0,2 †	**0,050**
Serum insulin (mU/L)	7,3 ± 0,6	9,2 ± 0,6	19,6 ± 1,6 *** †	**<0,001**
HOMA-IR	1,8 ± 0,1	2,1 ± 0,1	5,0 ± 0,4 *** †	**<0,001**
Systolic BP (mmHg)	122 ± 2	135 ± 6	143 ± 6 *	0,066
Diastolic BP (mmHg)	72 ± 1	81 ± 4	86 ± 5	0,108
$VO2_{peak}$ (ml*min^{-1}*kg^{-1} (FFM))	62 ± 3	48 ± 2 **	48 ± 1 **	**0,002**
W_{peak} (Watt*kg^{-1} (FFM))	4,9 ± 0,1	3,7 ± 0,2 ***	3,4 ± 0,1 ***	**<0,001**

	Obese insulin sensitive (n = 8)		Obese insulin resistant (n = 9)				
	Pre intervention	Post intervention	Pre intervention	Post intervention	P_{Time}	P_{Group}	$P_{Time*Group}$
Body weight (kg)	104,5 ± 3,2	102,8 ± 2,6	110,6 ± 5,1	108,3 ± 5,0	**0,002**	0,360	0,585
Body mass index (kg/m²)	32,7 ± 0,5	32,2 ± 0,3	34,1 ± 0,8	33,3 ± 0,8	**0,002**	0,225	0,550
Waist circumference (cm)	108,8 ± 1,6	108,8 ± 1,5	117,6 ± 3,3	113,5 ± 1,3	**0,039**	**0,004**	0,076
Fat mass (kg)	31,22 ± 1,91	29,11 ± 1,69	35,00 ± 1,92	33,07 ± 2,02	**<0,001**	0,168	0,833
Fat percentage (%)	31,6 ± 1,1	30,0 ± 1,0	33,7 ± 1,1	32,8 ± 1,2	**<0,001**	0,150	0,267
Fat free mass (kg)	66,8 ± 1,4	67,2 ± 1,3	68,5 ± 3,1	67,5 ± 3,3	0,377	0,779	**0,037**
$VO2_{peak}$ (ml*min^{-1}*kg^{-1} (FFM))	49 ± 2	53 ± 1	48 ± 1	55 ± 2	**0,012**	0,800	0,521
W_{peak} (Watt*kg^{-1} (FFM))	3,8 ± 0,2	4,1 ± 0,1	3,4 ± 0,1	4,0 ± 0,1	**<0,001**	0,382	0,069

Data are mean ± SE. * Significantly different from lean group p < 0,05; ** p < 0,01; *** p < 0,001; † Significantly different from obese insulin sensitive group (p < 0,05). FFM: fat free mass;$VO2_{peak}$: maximum oxygen uptake; W_{peak}: maximum power output.

Figure legends

Figure 1. Plasma glycerol, FFA, glucose, lactate, ANP, adrenalin, noradrenalin and serum insulin concentrations at rest, during exercise and recovery. Systemic glycerol **(A),** FFA **(B)**, glucose **(C)**, lactate **(D)**, ANP **(E)**, insulin **(F)**, adrenalin **(G)** and noradrenalin **(H)** responses in lean (white circles; n=10), obese insulin sensitive (white squares; n=10) and obese insulin resistant (black triangles; n=10) individuals. Data are presented as mean ± SEM. Group effects were tested with a one-way ANOVA with Bonferroni post-hoc. P_{ANOVA} values represent differences in exercise-induced systemic responses between groups, expressed as $AUC_{0\text{-}60}$ (area under the curve during exercise (from time point 0 till 60min)).

Figure 2. Changes in subcutaneous adipose tissue extracellular glycerol concentration and adipose tissue blood flow indices. Subcutaneous adipose tissue ethanol ratio's in lean (n=10) (**A**), obese insulin sensitive (n=8) (**B**) and obese insulin resistant (n=8) (**C**) individuals. Changes in extracellular glycerol concentration in lean (n=10) (**D**), obese insulin sensitive (n=8) (**E**) and obese insulin resistant (n=7) (**F**) individuals at rest, during exercise and recovery in control probe (white circles) and the probe perfused with phentolamine and propranolol (black squares). Mean changes in subcutaneous adipose tissue extracellular glycerol concentration during 1 h of low-intense exercise (40% VO_{2peak}). Changes were calculated by the difference between the mean glycerol concentrations during exercise and the baseline concentration and investigated using two-way repeated-measures ANOVA (p_{group}=0.009, $p_{treatment}$=0.069, $p_{treatment*group}$=0.035) (**G**). Data are presented as mean ± SEM. * Significantly different from the control probe ($p<0.05$) (A-F) or compared to the control probe from the lean group (G); N.S.: not significant.

Figure 3. Exercise training-induced changes in systemic plasma glycerol, FFA, glucose, lactate, ANP, adrenalin, noradrenalin and serum insulin in obese individuals. Systemic glycerol **(A-B),** FFA **(C-D)**, glucose **(E-F)**, lactate **(G-H)**, insulin **(I-J)**, ANP **(K-L)**, adrenalin **(M-N)** and noradrenalin **(O-P)** of obese insulin sensitive (n=8) and obese insulin resistant individuals (n=9) at baseline (white circles) and after 12 weeks of exercise training intervention (black squares). Data are presented as mean ± SEM. Exercise-induced responses were expressed as $AUC_{0\text{-}60}$ (area under the curve during exercise (from time point 0 till 60min)). Intervention effects were tested with two-way ANOVA with Bonferroni post-hoc.

Figure 4. Exercise training-induced changes in subcutaneous adipose tissue extracellular glycerol concentration and adipose tissue blood flow indices in obese insulin resistant individuals. Subcutaneous adipose tissue ethanol ratio (**A**) and extracellular glycerol concentration (**B**) in the obese insulin resistant individuals (n=8) at rest, during exercise and recovery after 12 weeks of exercise training in control probe (white circles) and the probe perfused with phentolamine and propranolol (black squares). Data are presented as mean ± SEM. $AUC_{0\text{-}60}$ (area under the curve during exercise (from time point 0 till 60min)) was used for statistical analysis (paired t-test).