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Short communication

Lipolysis in skeletal muscle is rapidly regulated by low physiological doses of insulin

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Abstract

Aims/hypothesis. Both patients with Type II (non-insulin-dependent) diabetes mellitus and normoglycaemic, insulin resistant subjects were shown to have an increased lipid content in skeletal muscle, which correlates negatively with insulin sensitivity. Recently, it was shown that during a hyperinsulinaemic euglycaemic clamp interstitial glycerol was reduced not only in adipose tissue but also in skeletal muscle. To assess whether lipolysis of muscular lipids is also regulated by low physiological concentrations of insulin, we used the microdialysis technique in combination with a 3-step hyperinsulinaemic glucose clamp.
Methods. Nineteen lean, healthy subjects (12 m/7 f) underwent a glucose clamp with various doses of insulin (GC I = 0.1, GC II = 0.25 and GC III = 1.0mU · kg^{-1} · min^{-1}). Two double lumen microdialysis catheters each were inserted in the paraumbilical subcutaneous adipose tissue and in skeletal muscle (tibialis anterior) to measure interstitial glycerol concentration (index of lipolysis) and ethanol outflow (index of tissue flow).
Results. During the different steps of the glucose clamp, glycerol in adipose tissue was reduced to 81 ± 7 % (GC I), 55 ± 8 % (GC II) and 25 ± 5 % (GC III), respectively, of basal. In contrast, glycerol in skeletal muscle declined to 73 ± 5 % (GC I) and to 57 ± 6 % (GC II) but was not further reduced at GC III. Tissue flow was higher in the skeletal muscle and remained unchanged in both compartments throughout the experiment.
Conclusion/interpretation. This study confirms the presence of glycerol release in skeletal muscle. Lipolysis in skeletal muscle and adipose tissue are suppressed similarly by minute and physiological increases in insulin but differently by supraphysiological increases. Inadequate suppression of intramuscular lipolysis resulting in increased availability of non-esterified fatty acids, could represent a potential mechanism involved in the pathogenesis of impaired glucose disposal, i.e. insulin resistance, in muscle. [Diabetologia (1999) 42: 1171–1174]

Keywords Skeletal muscle, adipose tissue, intramyocellular lipids, lipolysis, insulin resistance.

The relation between increased availability of non-esterified fatty acids (NEFA) and impaired muscle glucose disposal is well established for insulin resistant states. Increased lipolysis in subcutaneous and visceral adipose tissue is commonly assumed to be the source of these NEFAs [1, 2]. In addition to the well-known fat depots, however, muscle has been identified as tissue containing relevant amounts of lipids. These were shown to be located not only extramyocellularly but also intramyocellularly [2–4]. Moreover, a statistically significant correlation between the intramyocellular lipid content (by magnetic resonance spectroscopy) and insulin resistance (decreased metabolic clearance rate during a hyperinsulinaemic euglycaemic clamp) in normal glucose tolerant subjects was recently shown indicating an important role of muscular lipids for glucose homeostasis

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Abbreviations: GC, glucose clamp.

[3, 4]. Finally, lipolysis of muscular triglycerides was shown to be regulated by supraphysiological concentrations of insulin [5–7] making it a prime candidate for directly interfering with glucose disposal.

To evaluate in what way lipids in the muscle compartment might interfere with glucose disposal, it remains to be determined whether intramuscular lipid contents are also regulated by insulin concentrations in the physiologic range. Therefore, to assess the sensitivity of muscular lipolysis to physiologic increments in insulin we measured interstitial glycerol release in muscle during a 3-step hyperinsulinaemic euglycaemic clamp and compared it with simultaneously determined glycerol release in adipose tissue using the microdialysis technique.

Subjects and methods

Subjects. In total 19 healthy subjects [7 women 12 men; BMI 24.0 ± 4.8 kg/m^2; age 27.4 + 5.2 years (means + SD)] who did not take any medication participated in the study. Of the subjects 12 had a family history of Type II (non-insulin-dependent) diabetes mellitus; all participants had a normal oral glucose tolerance test according to WHO criteria. All volunteers had given written informed consent, and the study was approved by the ethics committee of the University of Tübingen.

Microdialysis procedure. Interstitial glycerol which is neither taken up nor metabolised to a major extent by adipose tissue and skeletal muscle is generally accepted as a qualitative index of hydrolysis of lipids [5–7]. Therefore, changes in dialysate glycerol reflect changes in tissue lipolysis [5, 6].

Pilot studies showed that interstitial glycerol concentrations in skeletal muscle declined (variably) for as long as 4 h after insertion of the catheter. Therefore, all subjects reported to the metabolic unit the evening before the experiment, catheters were inserted and perfused during the night to achieve stable baseline conditions before the start of the experimental procedure in the morning of the following day. After application of local anaesthesia two custom-made double lumen microdialysis catheters (CMA 60; CMA, Stockholm, Sweden) each were inserted in the periumbilical subcutaneous adipose tissue and in the tibialis anterior muscle, respectively, connected to the perfusion pumps (CMA 102 or 106, CMA, Stockholm, Sweden) and perfused with a modified Krebs-Henseleit buffer at 0.3 μl/min [7].

In a separate set of experiments we determined recovery of glycerol using [d_5] glycerol following the procedure described previously [6] in the tibialis anterior and adipose tissue in three subjects during an 8-h saline infusion and subsequently during hyperinsulinaemia ($1\ mU \cdot kg^{-1} \cdot min^{-1}$ for 2 h). At the flow rate of 0.3 μl/min which was also used in the metabolic study we found recoveries of 96 ± 3 % and 61 ± 10 % in adipose tissue and the tibialis anterior muscle, respectively. Thus, in adipose tissue nearly absolute tissue concentrations are measured, but in tibialis anterior dialysate glycerol underestimates the interstitial concentrations. Furthermore, recovery did not change over time and was not influenced by insulin.

Dialysate was collected every 20 min for analysis of glucose and glycerol. For the estimate of tissue blood flow, the second catheter was perfused with a perfusion fluid supplemented with 50 mmol/l ethanol to assess the ethanol outflow: inflow ratio [6, 7]. The catheters were perfused with 3.0 μl/min; dialysate was collected simultaneously with the metabolic analyses. The ethanol outflow: inflow ratio was used as an indirect estimate of tissue blood flow, with lower ratios indicating a higher flow and vice versa.

Three-step hyperinsulinaemic euglycaemic glucose clamp. In the morning of the experiment, two teflon catheters were inserted, one into the antecubital vein for infusion of glucose and insulin and another one in a retrograde fashion in a dorsal hand vein, which was placed under a heating device for sampling of arterialized blood. After collection of 3 baseline samples, a 3-step hyperinsulinaemic euglycaemic clamp was started. Each step lasted 120 min; at step I, insulin was infused at a rate of $0.1\ mU \cdot kg^{-1} \cdot min^{-1}$ (GC I), at step II with a rate of $0.25\ mU \cdot kg^{-1} \cdot min^{-1}$ (GCII) and at step III with $1.0\ mU \cdot kg^{-1} \cdot min^{-1}$ (GCIII). A variable infusion of exogenous glucose (dextrose 20–40 %) was adjusted to maintain euglycaemia within a narrow range (cv < 5 % for each step).

Blood sampling procedure. At baseline, arterialized blood was drawn for the determination of glucose, NEFA, glycerol, and insulin. During the glucose clamp whole blood glucose was monitored every 5–10 min with a bedside glucose analyser (glucose-oxidase method; Yellow Spring Instruments, USA). Every 20 min plasma was collected for the analysis of NEFA, glycerol and insulin. Due to the dead space of the microdialysis catheter, plasma samples were collected with a lag time of 10 min, i.e. in the middle of each microdialysis sampling interval, to synchronize sampling from blood and tissue.

Analytical procedures. Analyses were done with commercially available test kits for insulin (MEIA, Abbott, Wiesbaden, Germany), non-esterified fatty acids (Wako chemicals, Neuss, Germany), glycerol (Sigma diagnostics, Deisenhofen, Germany) and ethanol (ALC, DuPont, Wilmington, Del. USA). Dialysate concentrations of glucose and glycerol were measured in a CMA-600-bedside analyser (kindly provided by Roche-diagnostics) with commercially available test kits (CMA, Stockholm, Sweden), which previously have been shown to correlate closely with conventional methods [6].

Calculations and statistical analysis. The amount of exogenous glucose necessary to maintain euglycaemia during the last 40 min of each step is given as the glucose infusion rate. Interstitial glycerol concentrations reached an apparent steady state at the end of each insulin step; therefore, the mean value of the last 40 min at each step was used to calculate the difference from the previous step.

Due to the differences in recoveries, dialysate glycerol concentrations are used only as a qualitative index of lipolysis in muscle and adipose tissue. Therefore, changes in both interstitial and serum glycerol concentrations as well as serum NEFA concentrations are shown as per cent change from baseline (i.e. 100 %). Data are given as means ± SEM unless otherwise stated. Changes over time, i.e. basal and at the 3 steps of the hyperinsulinaemic euglycaemic clamp and differences between compartments (muscle vs adipose tissue) were calculated by a full factorial analysis of variance (ANOVA) with repeated measures design and the factors step and compartment. Direct comparisons between compartments at individual steps were made using the paired, two-tailed student's t test. The software package SPSS/PC + (SPSS, Chicago, Ill., USA) was used for statistical analysis. A p value of less than 0.05 was considered to be statistically significant.

Table 1. Metabolic variables at baseline and during the three-step hyperinsulinaemic euglycaemic clamp

	Baseline	GC I	GC II	GC III
Blood glucose (mmol · l^{-1})	4.88 ± 0.1	4.78 ± 0.1	4.88 ± 0.1	4.91 ± 0.1
Serum insulin (pmol · l^{-1})	49.8 ± 14.4	63.0 ± 9.6[a]	123.0 ± 15.6[a]	381.5 ± 23.4[a]
Glucose infusion rate (mg · kg^{-1} · min^{-1})	–	–	2.0 ± 0.2[a]	7.8 ± 0.5[a]
Serum NEFA (mmol · l^{-1})	0.46 ± 0.05	0.32 ± 0.03[a]	0.12 ± 0.02[a]	0.04 ± 0.01[a]
Serum glycerol (μmol · l^{-1})	62 ± 6.5	45 ± 3.3[a]	37 ± 3.3[a]	32 ± 3.3[a]
Dialysate glycerol-skeletal muscle (μmol · l^{-1})	42 ± 5.4[b]	30 ± 4.3[a,b]	22 ± 4.3[a,b]	22 ± 4.3[b]
Dialysate glycerol-adipose tissue (μmol · l^{-1})	206 ± 20.6	152 ± 17.4[a]	103 ± 18.5[a]	52 ± 8.7[a]
Ethanol outflow : inflow ratio skeletal muscle	0.24 ± 0.03[b]	0.23 ± 0.02[b]	0.22 ± 0.03[b]	0.23 ± 0.03[b]
Ethanol outflow : inflow ratio adipose tissue	0.47 ± 0.03	0.48 ± 0.03	0.47 ± 0.03	0.47 ± 0.03

Values at each step represent means of three points in time during the last 40 min of each step.

[a] p vs previous step < 0.05
[b] p vs adipose tissue < 0.05

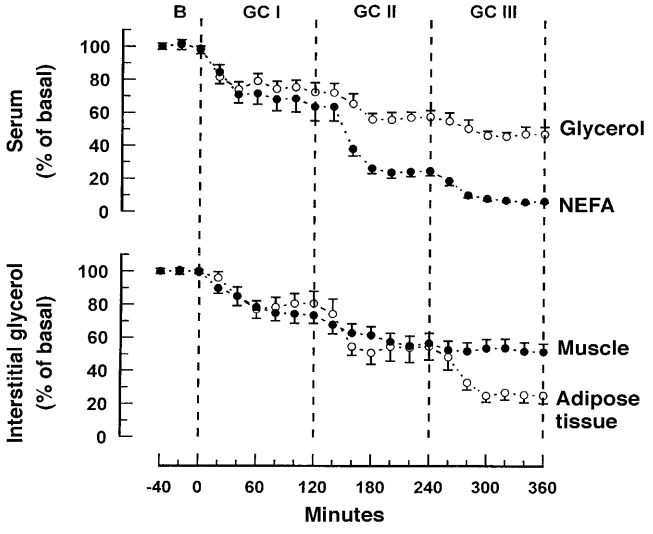

Fig. 1. Change of serum NEFAs and serum glycerol (upper panel) and interstitial glycerol in adipose tissue and skeletal muscle (lower panel) at baseline and during the three-step hyperinsulinaemic euglycaemic clamp. Values are expressed as percentage of mean baseline. Differences between skeletal muscle and adipose tissue at each step (t test) were $p = 0.39$ at GC I, $p = 0.82$ at GC II and $p < 0.001$ at GC III (B, baseline; GC I-III, steps I-III of the glucose clamp; for details see methods)

Results

Before the start of the glucose clamp, all serum and interstitial metabolite concentrations had achieved an apparent plateau (–40 to 0 min). Glucose concentrations were similar in the three different compartments at baseline and during the glucose clamp (data not shown). In contrast, glycerol concentrations were lowest in tibialis anterior muscle dialysate, intermediate in plasma and highest in adipose tissue dialysate (Table 1, $p < 0.002$). Due to the recovery of about 60 % in skeletal muscle, dialysate concentrations underestimate, however, true interstitial glycerol concentrations. During the last 40 min of each step, a metabolic steady state for glycerol was reached in skeletal muscle and adipose tissue as shown by the slope of the last 3 measurements over time not being statistically significantly different from zero (data not shown).

Alterations in NEFAs and glycerol during the 3-step hyperinsulinaemic euglycaemic clamp (Table 1, Fig. 1). The lowest insulin infusion, glucose clamp I (GC I) which increased plasma insulin slightly, induced a large decrease in serum NEFA, glycerol and also a rapid and pronounced fall in tissue glycerol in both compartments ($p < 0.005$). When plasma insulin was further increased (GC II), serum NEFA and glycerol continued to decrease ($p < 0.005$) and interstitial glycerol declined further in both tissues ($p < 0.005$). Serum glucose remained constant in all 3 compartments during infusion of exogenous glucose. At GC III, insulin infusion resulted in supraphysiological insulin concentrations. Plasma NEFA were almost completely suppressed ($p < 0.005$) but serum glycerol was not lowered any further. Interstitial glycerol in the tibialis anterior remained constant but continued to decrease in the adipose tissue to 25 ± 4.8 % of baseline ($p < 0.005$).

Comparison adipose tissue with skeletal muscle (Table 1). Absolute dialysate glycerol concentration was higher in adipose tissue at baseline and remained higher than in skeletal muscle at all time points ($p < 0.002$). Although at the first two steps, the decline in interstitial glycerol was similar, at GC III, there was a greater decrease in skeletal muscle than in adipose tissue ($p < 0.04$).

Interstitial glycerol release. Tissue blood flow as estimated by the ethanol outflow: inflow ratio was higher in skeletal muscle (Table 1). Hyperinsulinaemia did not induce any changes in the ethanol outflow: inflow ratio in any compartment. Thus, the variation of dialysate glycerol concentration during the 3-step glucose clamp most likely reflected the alterations of its release by the different tissues.

Discussion

In this study we have shown an appreciable release of glycerol not only in adipose tissue but also in skeletal muscle which confirms previous reports using similar techniques [5–7]. Although earlier studies examined

the effect of a standard hyperinsulinaemic euglycaemic clamp on lipolysis in adipose tissue and skeletal muscle [5–7], we also used lower, more physiological doses of insulin.

One important finding of this study is that minute increases in insulin concentrations well within the physiological range (10.5 and 20.5 $\mu U \cdot ml^{-1}$), promptly reduce interstitial glycerol to the same degree in both tissues. That lipolysis in muscle is at least as sensitively regulated by insulin as in adipose tissue points to the metabolic relevance of intramuscular lipids.

It is not known whether dialysate glycerol collected in skeletal muscle originates from adipose tissue located between the muscle fibres or from hydrolysis of intramyocellular lipids or both. Nevertheless, considering organ size and blood flow, lipids in the muscular compartment could be of relevance for muscle energy homeostasis regardless of the precise cellular origin. Non–esterified fatty acids derived from hydrolysis of muscular fat would be immediately available in skeletal muscle to decrease skeletal muscle glucose uptake, glycogen synthesis and glucose oxidation [1, 2, 8–10]. Conceivably, inadequate suppression of intramuscular lipolysis and excessive delivery of NEFAs could impair uptake and disposal of glucose in an autocrine or paracrine fashion representing a potential mechanism for the development of insulin resistance. Therefore, it is tempting to speculate that a dysregulation of lipolysis of the recently reported excessive intramyocellular lipids in insulin-resistant subjects could be responsible for their diminished insulin-stimulated glucose uptake [3, 4].

Secondly, we observed a statistically significant difference between both tissues in the relative decline of interstitial glycerol which, at the highest insulin concentration (63.5 $\mu U \cdot ml^{-1}$), continued to decrease in adipose tissue but remained unchanged in skeletal muscle. Thus, our data suggest differences in the regulation or the insulin sensitivity of lipolysis in skeletal muscle and adipose tissue. These observations are at variance with previous clamp studies achieving similar insulin concentrations [6, 7] but finding no difference between the two tissues. A possible explanation for this discrepancy are the differences in the type of muscle investigated (gastrocnemius vs tibialis in this study) and the experimental design used (instantaneous supraphysiological hyperinsulinaemia vs two preceding steps of mild and moderate hyperinsulinaemia in this study). Nevertheless, the concept of differential regulation is supported by the different phosphodiesterase subtypes [7] and different beta-adrenergic receptor subtypes [10] in muscle and adipose tissue, respectively, to be involved in inhibition or stimulation of lipolysis or both.

At the end of the 6-h, three-step euglycaemic hyperinsulinaemic clamp, dialysate glycerol concentration was not completely suppressed. This observation was made previously during a standard 2-h (insulin infusion of 1 $mU \cdot kg^{-1} \cdot min^{-1}$) [6, 7] and also during a 3-h hyperinsulinaemic clamp using even higher insulin infusion rates (3 $mU \cdot kg^{-1} \cdot min^{-1}$) [5]. There is no satisfactory explanation for this phenomenon. Possible, underlying mechanisms include the presence of lipolysis in spite of hyperinsulinaemia, a certain proportion of lipolysis which is not regulated by insulin and other metabolic sources of glycerol such as glycolysis [5–7].

Our study confirms the presence of glycerol release in skeletal muscle. Lipolysis in skeletal muscle and adipose tissue are suppressed similarly by minute and physiological increases in insulin but differentially by supraphysiological increases. Inadequate suppression of intramuscular lipolysis possibly represent a potential mechanism involved in the pathogenesis of impaired glucose disposal, i.e. insulin resistance, in muscle.

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