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ARTICLE

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Heterogeneity in limb fatty acid kinetics in type 2 diabetes

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Abstract *Aims/hypothesis:* In order to test the hypothesis that disturbances in skeletal muscle fatty acid metabolism with type 2 diabetes are not equally present in the upper and lower limbs, we studied fatty acid kinetics simultaneously across the arm and leg of type 2 diabetic patients (n=6) and matched control subjects (n=7) for 5 h under baseline conditions and during a 4-h hyperinsulinaemic–euglycaemic clamp. *Methods:* Limb fatty acid kinetics was determined by means of continuous [U-^{13}C]palmitate infusion and measurement of arteriovenous differences. *Results:* The systemic palmitate rate of appearance was 3.6±0.4 and 2.7±0.3 μmol·kg lean body mass^{-1}·min^{-1} and decreased during the clamp by 26% (p=0.04) and 43% (p<0.01) in the diabetic patients and in the control subjects respectively. At baseline, palmitate uptake across the arm was similar in the two groups, whereas leg palmitate uptake was lower than in the arm in the diabetic patients. During the clamp, palmitate uptake decreased in the arm (−48%, p=0.02) and the leg (−38%, p=0.04) of the control subjects, whereas it decreased in the arm (−30%, p=0.04) but not in the leg of the diabetic patients. Similarly, during the clamp palmitate release was substantially suppressed in the arm (−47%, p<0.01) and the leg of the control subjects (−45%, p<0.01), but only in the arm of the diabetic patients (−45%, p<0.01). *Conclusions/interpretation:* The present data indicate that type 2 diabetes is characterised by heterogeneity in the dysregulation of skeletal muscle fatty acid metabolism, with only the leg, but not the arm, showing an impairment of fatty acid kinetics at baseline and during a hyperinsulinaemic–euglycaemic clamp causing a physiological increase in insulin concentration.

Keywords Diabetes · Fatty acid · Limb · Skeletal muscle

Abbreviations FAT: fatty acid translocase · R_a: rate of appearance · R_d: rate of disappearance · [U-^{13}C]palmitate: ^{13}C uniformly labelled palmitate

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Introduction

Disturbances of fatty acid metabolism may play a role in the development of insulin resistance and type 2 diabetes. In the postabsorptive state type 2 diabetic patients have an increased plasma fatty acid concentration [1, 2], which may interfere with glucose homeostasis/metabolism. In healthy volunteers it has been shown that an elevation of plasma fatty acid levels for some hours induced insulin resistance [3–5] and that a reduction in fatty acid level reduced the severity of insulin resistance in type 2 diabetic patients [6].

Skeletal muscle is an important tissue for the maintenance of glucose and fatty acid homeostasis in the body, and being a prime target for insulin action is therefore linked to insulin resistance. Reduced plasma fatty acid uptake and oxidation by the leg [7] and the arm [8, 9] have been observed in type 2 diabetics as compared to healthy individuals. Moreover, the relationship between intramuscular lipid accumulation and insulin resistance has been described [10, 11]. Physical inactivity or muscle disuse is recognised as one of the risk factors for the development of insulin resistance and type 2 diabetes [12, 13]. Against this, it has been shown that exercise training can reduce insulin resistance in the leg of diabetic patients [14, 15]. Recently, evidence has been presented for heterogeneity towards lipolysis in human skeletal muscles [16]. Given the different roles of arm and leg muscles and their different activation in daily life, it is possible that the observed disturbances in skeletal muscle fatty acid kinetics in type 2 diabetes are not equal in skeletal muscles of the arms and

legs. To test this hypothesis, we investigated limb fatty acid kinetics by means of a combination of stable isotope dilution and an arterial–venous balance technique applied to the arm and the leg of sedentary type 2 diabetic patients and matched healthy volunteers under baseline conditions and during a hyperinsulinaemic–euglycaemic clamp causing an increase in insulin well within the physiological range.

Subjects, materials and methods

Subjects

Six type 2 diabetic male volunteers and seven matched healthy volunteers (controls) participated in the study. The characteristics of the subjects are reported in Table 1. All subjects had had a stable body weight for at least the preceding 3 months and were not engaged in any regular strenuous physical activity or have a physically demanding job. Two of the diabetic patients were treated with diet only, one patient with diet and insulin, and three patients with diet and oral glucose-lowering agents (two patients with metformin and one patient with metformin and sulphonylureas). All medications were withheld 24 h before the experiments. None of the patients had health problems, apart from type 2 diabetes. None of the healthy control subjects were receiving medication and all had a normal oral glucose tolerance test. The patients and healthy volunteers were informed about the aim and the possible risks involved in the study and gave their written consent to participation. The study was conducted according to the Declaration of Helsinki and had been approved by the Ethical Committee of Copenhagen–Frederiksberg Communities.

Body composition assessment

Body composition was determined by dual-energy X-ray absorptiometry, which has been shown to provide a valid and reproducible estimation of limb fat and muscle mass [17]. Before each scan the machine was calibrated with phantoms of known composition.

Table 1 Subject characteristics

	Control subjects (n=7)	Type 2 diabetic subjects (n=6)
Age (years)	48±4	58±2[a]
Body weight (kg)	89±7	105±6
BMI (kg/m^{-2})	28±2	33±2
Percentage body fat	28±2	29±3
Lean body mass (kg)	61±4	70±3
Percentage leg fat	25±3	27±3
Percentage arm fat	25±2	27±3
Leg muscle mass (kg)	7.9±0.6	9.0±0.3
Arm muscle mass (kg)	3.3±0.2	3.9±0.2
Fasting glucose (mmol/l)	5.3±0.2	8.6±0.8[a]

Data are means±SE
[a]Significantly different from controls (p<0.05)

Experimental procedure

The type 2 diabetic patients and control subjects refrained from any of their ordinary physical activities during the 48 h preceding the trial. On the day of the experiment the subjects reported to the laboratory at 08.00 hours after an overnight fast (12 h). After 20 min of supine rest, Teflon catheters (20 G; Ohmeda, Wiltshire, UK) were inserted in the femoral artery and in the retrograde direction into the femoral vein of one leg using the Seldinger technique. In addition, a 16 G catheter was inserted into an antecubital vein of one arm and the tip of the catheter advanced into the subclavian vein until approximately 5 cm before the merger with the jugular vein. The positioning of the tip of the catheter was verified by X-ray. Furthermore, two catheters were placed in antecubital veins of the other arm for stable isotope, glucose and insulin infusion. Twenty minutes after the catheterisation procedure, baseline blood samples were taken and femoral and subclavian blood flow was measured by Doppler ultrasound [18]. Subsequently, a constant infusion of [U-^{13}C]palmitate (0.009 μmol·kg^{-1}·min^{-1}; Cambridge Isotope Laboratories, Andover, MA, USA) was started. The isotope infusion was continued for 9 h, during which time the subjects remained in the supine position. Blood sampling and blood flow measurements were performed every 60 min throughout the experiment. Before blood sampling, a pneumatic cuff was placed under the knee and around the wrist and inflated to a suprasystolic pressure to avoid shunting in the lower leg and in the hand [19]. Five hours after the start of the stable isotope infusion, an insulin infusion (1 pmol kg^{-1}·min^{-1}) was started and continued for the remaining 4 h of the study. During the hyperinsulinaemic clamp the arterial glucose concentration was maintained at the value recorded at the end of the baseline period (5.07±0.1 mmol/l in the control subjects and 6.86±1.0 mmol/l in the diabetic patients) by infusion of a 20% glucose solution. The [U-^{13}C]palmitate infusion rate was reduced by 50% at the start of the hyperinsulinaemic–euglycaemic clamp. Arterial glucose and potassium concentrations were measured every 10 min during the clamp period (ABL 715; Radiometer, Copenhagen, Denmark) and potassium was infused to maintain the plasma potassium concentration.

Analytical procedures

Blood for the measurement of substrate concentrations and isotopic enrichment was collected in prechilled tubes containing 0.3 M EDTA (10 μl ml^{-1} blood) and immediately centrifuged for 10 min at 4°C. The plasma obtained was immediately frozen and stored at −80°C until analysis. Plasma insulin was determined with an ELISA kit (Dako, Glostrup, Denmark). Extraction of plasma fatty acid for the

determination of palmitate concentration and isotopic enrichment was performed according to Patterson and colleagues [20]. Palmitate concentration was measured by gas chromatography with FID (Autosystem XL; Perkin Elmer, Northwalk, CT, USA), using heptadecanoic acid as an internal standard. Plasma palmitate ^{13}C enrichment was measured by gas chromatography-combustion isotope ratio mass spectrometry (GC-C-IRMS, Hewlett Packard 5890, Finnigan GC combustion III, Finnigan Delta^{plus}; Finnigan MAT, Bremen, Germany) as described previously [21]. Palmitate enrichment was corrected by a factor of 17/16 to account for the extra methyl group of the methyl palmitate derivative.

Calculations

Whole-body palmitate kinetics The whole-body rate of appearance (R_a) of palmitate in plasma, which in steady-state conditions is equal to the rate of disappearance (R_d), was calculated by dividing the tracer infusion rate by the arterial plasma enrichment (Steele's equation for steady-state conditions [22]).

Limb substrate balance and kinetics Net palmitate balance across the leg and the arm was calculated by multiplying the arterial–venous concentration difference by plasma flow, calculated as blood flow×(1−haematocrit). The limb fractional extraction was calculated as

$$\text{fractional extraction} = \frac{(C_a \times E_a) - (C_v \times E_v)}{C_a \times E_a}$$

where C_a and E_a, and C_v and E_v are the concentration and the tracer enrichment of palmitate in the artery and the femoral/subclavian vein respectively. The limb palmitate uptake was calculated as

$$\text{uptake} = \text{fractional extraction} \times C_a \times \text{plasma flow}$$

and the limb release as

$$\text{release} = \text{uptake} - \text{net balance}.$$

Statistical analysis

Data are expressed as means±SE. The data are presented as average values of the 2–5 h (Basal) and 6–9 h (Insulin) periods, when an apparent isotopic and physiological steady state was achieved. Differences between the average values of the two periods, groups and limbs were determined by two-way repeated-measures analysis of variance (ANOVA). When ANOVA revealed a significant effect, a *t*-test was used to determine where the difference occurred. When the comparison between limbs was not applicable, differences between groups were determined with the unpaired *t*-test, whereas the effect of the clamp was determined with the paired *t*-test. Statistical significance was accepted at a p value of less than 0.05.

Results

Arterial and systemic palmitate rate of appearance (R_a) The arterial palmitate concentration was similar in diabetic patients and control subjects during the last 3 h of the baseline and hyperinsulinaemic–euglycaemic clamp period and was substantially decreased during the clamp (Fig. 1). Baseline palmitate R_a was 3.6±0.4 and 2.7±0.3 μmol·kg lean body mass^{-1}·min^{-1} in the diabetic patients and in the control subjects respectively (Fig. 1). Palmitate R_a decreased substantially during the hyperinsulinaemic–euglycaemic clamp in the control subjects (−43%, $p<0.01$) and the diabetic patients (−26%, $p<0.05$).

Limb palmitate kinetics Under baseline conditions a net palmitate uptake was observed across the leg, whereas a net palmitate release was observed across the arm in both

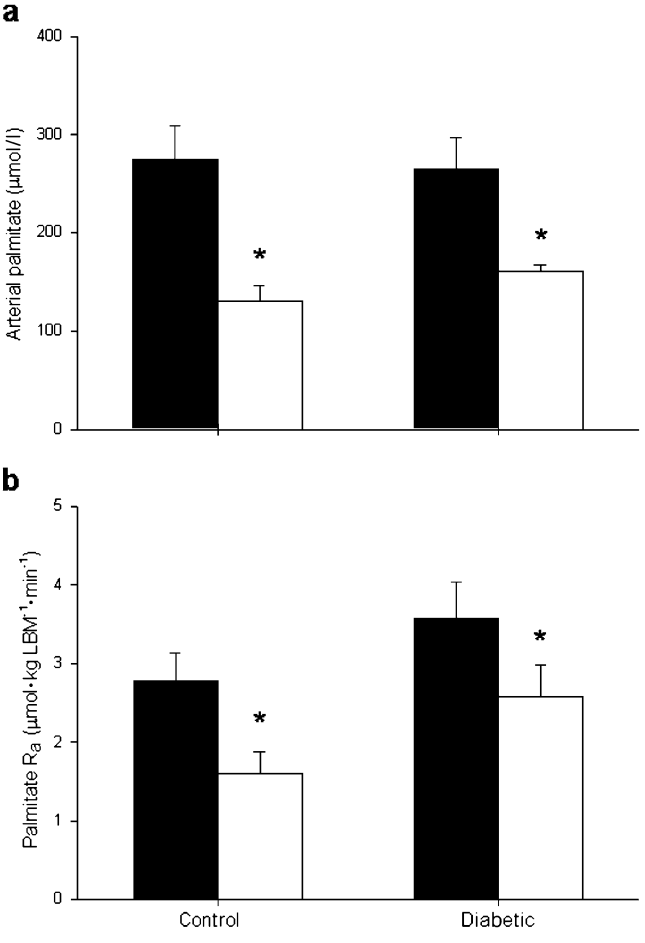

Fig. 1 Arterial palmitate and systemic palmitate rate of appearance (R_a) in control subjects and type 2 diabetic patients. **a** Arterial palmitate concentration and **b** systemic palmitate R_a in control subjects and in type 2 diabetic patients during the baseline (*basal, filled bars*) and hyperinsulinaemic–euglycaemic clamp (*insulin, open bars*) periods. *$p<0.05$ for difference from basal

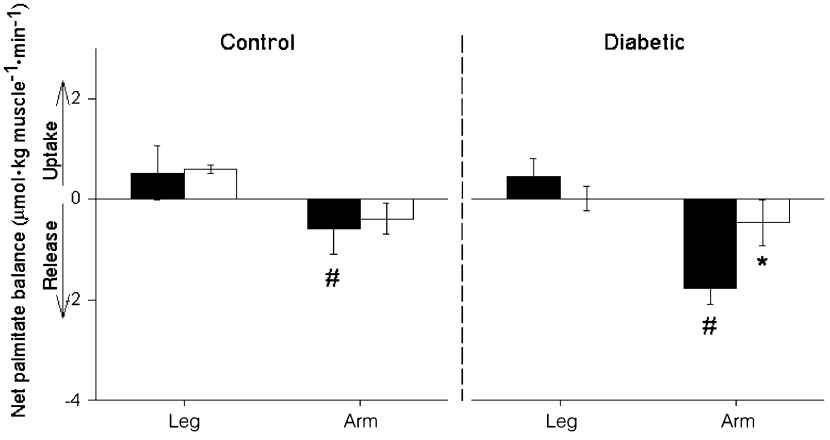

Fig. 2 Limb net palmitate balance (uptake and release) in control subjects and type 2 diabetic patients during the baseline (*basal, filled bars*) and hyperinsulinaemic–euglycaemic clamp (*insulin, open bars*) periods. **p*<0.05 for significant difference from basal; #*p*<0.05 for significant difference from leg

groups (Fig. 2). The unidirectional palmitate uptake was normalised for the limb muscle mass and palmitate release for the limb fat mass in order to compare arm and leg kinetics. In control subjects, palmitate uptake was not significantly different between the limbs during the baseline period and decreased similarly during the clamp (−48% and −38% for the arm and the leg, respectively). However, in the diabetic patients palmitate uptake by the leg was lower than that by the arm ($p<0.05$) (Fig. 3). During the hyperinsulinaemic–euglycaemic clamp, palmitate uptake was substantially decreased in the arm of the diabetic patients compared with baseline (−30%, $p=0.02$), whereas it was unchanged in the leg. Leg and arm blood flow was not significantly influenced by insulin infusion (Table 2).

The unidirectional palmitate release is a dependent variable of the tracer-determined palmitate uptake and the net palmitate balance across the limbs. Palmitate release was higher in the arm than in the leg under baseline conditions and during the clamp in control and diabetic subjects (Fig. 3). During the hyperinsulinaemic–euglycaemic clamp a substantial decrease in arm palmitate release was observed in both groups. In contrast, and similarly to what

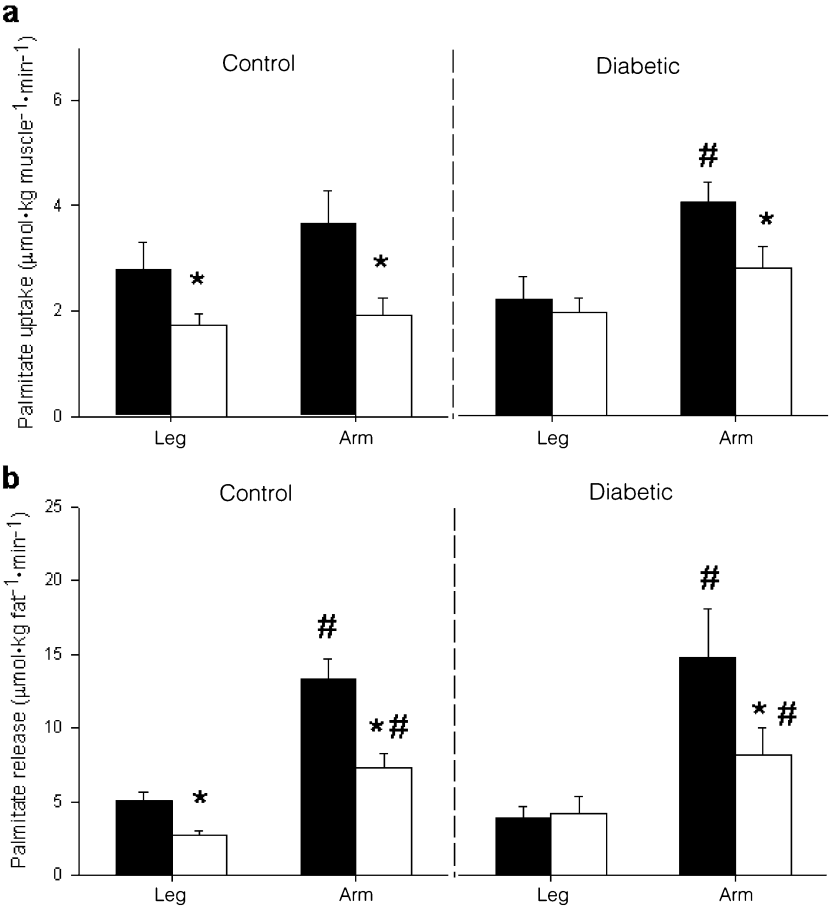

Fig. 3 Unidirectional limb palmitate uptake and release in control subjects and type 2 diabetic patients. **a** Limb unidirectional palmitate uptake (expressed per kg muscle mass) and **b** release (expressed per kg fat mass) during the baseline (*basal, filled bars*) and hyperinsulinaemic–euglycaemic clamp (*insulin, open bars*) periods. * *p*<0.05 for significant difference from basal; #*p*<0.05 for significant difference from leg

Table 2 Average blood flow and limb palmitate fractional extraction during the basal period and during the clamp period

		Control		Diabetic	
		Leg	Arm	Leg	Arm
Blood flow (l/min)	Basal	0.447±0.065	0.281±0.033[a]	0.363±0.038	0.343±0.050
	Insulin	0.433±0.059	0.278±0.030[a]	0.387±0.052	0.337±0.036
Palm FE (%)	Basal	34.6±5.6	30.8±4.2	35.0±4.2	27.3±3.3
	Insulin	50.3±3.7[b]	35.6±4.8	44.1±5.4	25.5±5.7

Data are means±SE
[a]Significantly different from leg ($p<0.05$)
[b]Significantly different from basal ($p<0.05$)

was observed for palmitate uptake, during the clamp palmitate release was unchanged in the leg of the diabetic patients but substantially suppressed in the leg of the control subjects (−47%, $p<0.01$).

Contribution of limb palmitate uptake/release to systemic palmitate R_d/R_a Under baseline conditions the fractional contribution of palmitate uptake by the arm to systemic palmitate R_d was similar in control subjects and diabetic patients, whereas the contribution of the leg was substantially lower in diabetic patients during the baseline and clamp period (Fig. 4).

Insulin concentration and plasma palmitate enrichment Arterial plasma insulin concentration during the last 3 h of the baseline period averaged 39±12 and 68±14 pmol/l in the control subjects and the diabetic patients, respectively. During the hyperinsulinaemic–euglycaemic clamp the insulin concentration increased to 81±11 and 141±24 pmol/l in the control subjects and the diabetic patients, respectively. The reduction of the [U-^{13}C]palmitate infusion rate at the beginning of the clamp period successfully minimised the changes in palmitate enrichment expected from the effect of insulin infusion (Fig. 5).

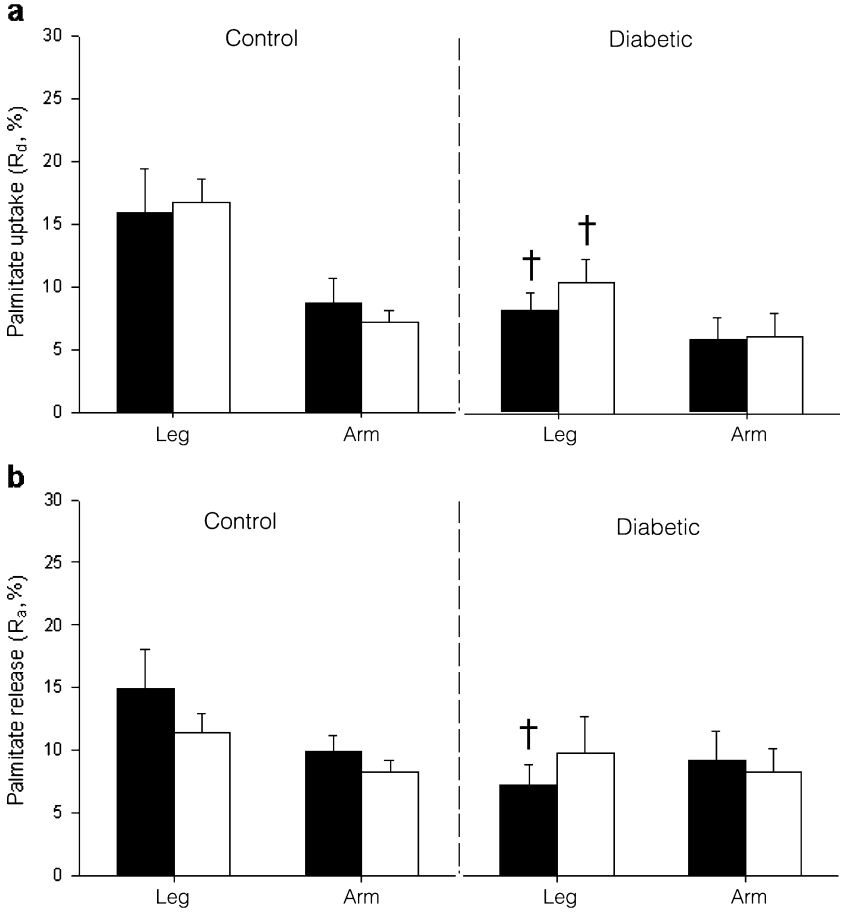

Fig. 4 Limb fractional contribution to systemic palmitate turnover in healthy control subjects and type 2 diabetic patients. Limb fractional contribution to systemic palmitate R_d (**a**) and R_a (**b**) in control subjects and type 2 diabetic patients during the baseline (*basal, filled bars*) and hyperinsulinaemic–euglycaemic clamp (*insulin, open bars*) periods. †$p<0.05$ for significant difference from control

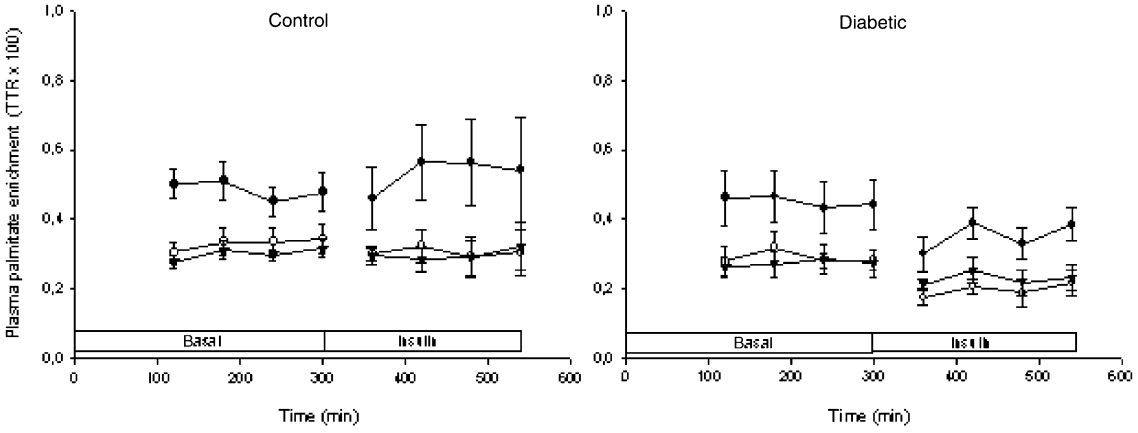

Fig. 5 Palmitate enrichment in the femoral artery (*closed circle*), femoral vein (*open circle*) and subclavian vein (*inverted triangle*) plasma in control subjects and type 2 diabetic patients during the baseline (basal) and the hyperinsulinaemic–euglycaemic clamp (insulin) periods

Discussion

The present study investigated arm and leg fatty acid kinetics in postabsorptive healthy volunteers and diabetic patients under baseline conditions and during a hyperinsulinaemic–euglycaemic clamp. The main findings were that: (1) palmitate uptake is similar in arm and leg of healthy individuals under baseline conditions and during a hyperinsulinaemic–euglycaemic clamp; (2) palmitate release, indicative of lipolysis, is substantially higher in the arm than in the leg; and (3) during a hyperinsulinaemic–euglycaemic clamp fatty acid kinetics is similar in the arm but markedly different in the leg in diabetic patients compared with healthy individuals. Thus, our hypothesis that disturbances in skeletal muscle fatty acid kinetics in type 2 diabetes are not equal in arm and leg muscles was confirmed.

In the present study, fatty acid kinetics was simultaneously determined for the arm and leg in order to determine potential differences in limb fatty acid metabolism. Because of the importance of fully understanding the dysregulation of fatty acid metabolism in type 2 diabetes, we also determined the effect of insulin on limb fatty acid kinetics using a hyperinsulinaemic–euglycaemic clamp in healthy individuals and diabetic patients. Since insulin is one of the most potent antilipolytic hormones [23], a low dose of insulin was infused with the intention of inducing an increase in insulin levels well within the normal physiological range and to prevent the complete blockage of lipolysis. Type 2 diabetes has been shown to be associated with impaired suppression of fatty acid levels and fatty acid R_a by insulin [24, 25]. Accordingly, in the present study a tendency was observed for a less significant suppression of arterial palmitate concentration and systemic palmitate turnover rate in diabetic patients during the hyperinsulinaemic–euglycaemic clamp. This observation is also in agreement with the impaired postprandial suppression of systemic fatty acid turnover demonstrated in type 2 diabetic patients [26]. From the present study it is clear that the skeletal muscles of the leg more than those of the arm may play an important role in the reduced insulin sensitivity with regard to the systemic fatty acid turnover in type 2 diabetes, since no suppression was observed in palmitate uptake or release by the leg.

Palmitate uptake under baseline conditions, expressed per kilogram of muscle mass, was not significantly different between the upper and lower limbs in the control subjects, whereas it was lower in the leg of the diabetic patients. Reduced fatty acid uptake has been observed in the forearm [8] and in the leg [7, 27] of type 2 diabetic patients. A lower capacity for fatty acid uptake in the diabetic leg muscles is also in agreement with similar findings in human cultured vastus lateralis muscle cells [28]. However, subsequent data on human cultured myotubes from the vastus lateralis did not indicate substantial differences between myotubes from diabetic patients and matched controls [29]. The decrease of palmitate uptake with insulin has been reported previously for the leg [7, 30] and the arm [31] of healthy subjects. Nevertheless, when fatty acid levels were maintained during the insulin infusion, the fatty acid uptake by skeletal muscle was actually increased in rat muscle preparations [32, 33] and in cultured cells from the vastus lateralis muscle [28, 29]. These observations imply that the reduction in limb fatty acid uptake with insulin is caused more by reduced limb fatty acid availability than by lessened skeletal muscle insulin sensitivity. However, the present study clearly shows that, in spite of the markedly reduced palmitate availability, the palmitate uptake in the leg is maintained during insulin infusion in diabetic patients. This suggests an important role of insulin in the regulation of skeletal muscle fatty acid metabolism and a derangement of this regulation in type 2 diabetes, which is potentially responsible for the chronically elevated fatty acid levels. Fatty acid uptake in skeletal muscle is thought to occur, at least in part, as a protein-mediated process [34, 35]. In humans, plasma membrane fatty acid binding protein in the vastus lateralis muscle has been reported to be either diminished [8] or increased [36] in type 2 diabetes, whereas fatty acid translocase (FAT)/CD36 has been suggested to be unaltered [36]. To our knowledge, however, data on fatty acid transport proteins in arm muscles in humans are lacking. Moreover, in rat skeletal muscle, in-

sulin has been proved to translocate FAT/CD36 from the intracellular pool to the plasma membrane, causing increased fatty acid uptake. Interestingly, protein-mediated fatty acid transport in human cultured muscle cells from the vastus lateralis has been shown to be stimulated by insulin in non-diabetic but not in type 2 diabetic subjects [28]. In the light of the results of the present study, it would be of interest to investigate the possibility of a different effect of type 2 diabetes on protein-mediated fatty acid transport in arm and leg muscles under both basal and insulin-stimulated conditions.

The limbs clearly consist not solely of skeletal muscle but also of subcutaneous adipose tissue, adipose tissue interspersed within and around the muscle bundles, and lipid droplets within the myocytes. While it has been suggested that adipose tissue has a very limited contribution to palmitate uptake in volunteers in the postabsorptive state [21, 37, 38], this is not the case for palmitate release. Furthermore, heterogeneity in adipose tissue [39] and skeletal muscle [16] lipolysis has been reported. In addition, skeletal muscle and adipose tissue lipolysis can respond differently to insulin [40–43], also in relation to the insulin level [44, 45]. Taken together, these factors hamper a clear interpretation of palmitate release in the arm and leg of healthy individuals and diabetic patients. Nevertheless, in the present study the similarity between the groups in the fat contents of the arm and leg reinforces the validity of the observation of a disturbed response of palmitate release in the leg to insulin in diabetic patients. In fact, whatever the prevalent source of palmitate release, this was suppressed differently by insulin in diabetic patients, being reduced in the arm but not in the leg.

Physical inactivity or muscle disuse is linked to the development of insulin resistance and type 2 diabetes [12, 13], whereas exercise training can reduce insulin resistance [14, 15]. The present findings of a marked difference in limb palmitate kinetics between arm and leg might be interpreted from the perspective of a different activity pattern between the two extremities. It could be argued that in sedentary individuals and with ageing, the leg undergoes a relatively more pronounced decline in muscular activity due to reduced locomotion, whereas the arm maintains better its activity level because of greater involvement in the activities of daily living, thereby being less prone to the development of insulin resistance. Whether or not this speculative explanation is true, and in view of their large muscle content, it is clear that the legs should be a prime target in the strategy to prevent insulin resistance and development of type 2 diabetes.

In conclusion, our study indicates that there is heterogeneity in the dysregulation of skeletal muscle fatty acid metabolism in type 2 diabetes in terms of fatty acid kinetics and its sensitivity to insulin stimulation, which are different in the leg but similar in the arm of type 2 diabetic patients compared with non-diabetic matched control subjects. The present data warrant future studies to elucidate the mechanism of the different metabolic perturbation in the arm and leg muscles of patients with type 2 diabetes.

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