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# Glucose oversupply increases Δ9-desaturase expression and its metabolites in rat skeletal muscle

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## Abstract

*Aim/hypothesis.* Previous studies have shown that prolonged glucose infusion causes insulin resistance and triglyceride accumulation in rat skeletal muscle. In this study, we investigated a possible relationship between insulin resistance and the composition of different accumulated lipid fractions in rat skeletal muscle.
*Methods.* Continuous glucose infusion was carried out in rats for 7 days. Lipids were extracted from skeletal muscle, separated by thin layer chromatography and fatty acid composition of phospholipids, triglycerides, diglycerides, free fatty acids and cholesterol esters fractions was analysed by gas chromatography. Δ9-Desaturase mRNA was measured by real time polymerase chain reaction. The enzyme activity was measured in the microsomal fractions.
*Results.* Prolonged glucose infusion (5 days) increased the relative content of palmitoleic acid (16:1 N7) several-fold (2.3- to 5.8-fold) in four out of five lipid fractions and enhanced oleic acid (18:1 N9) two-fold in three lipid fractions suggesting increased Δ9-desaturase activity while the content of several polyunsaturated fatty acids was reduced. In parallel, Δ9-Desaturase mRNA contents and enzyme activities in skeletal muscle were increased 10-fold, 75-fold, 2.6-fold and 7.7-fold after 2 and 5 days of glucose infusion, respectively.
*Conclusion/interpretation.* Our results suggest that long-term glucose oversupply induces a rapid increase in Δ9-desaturase expression and enzyme activity in skeletal muscle which leads to fast and specific changes in fatty acid metabolism possibly contributing to the insulin resistance in this animal model.
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**Keywords** Hyperglycaemia, hyperinsulinaemia, insulin resistance, lipid fractions, fatty acid composition, stearoyl-CoA desaturase.

Increased intramyocellular fat accumulation coincides with insulin resistance in humans [1, 2, 3, 4, 5, 6, 7] and in rodents, [8, 9, 10]. It has been suggested that increased adipocyte mass and impaired insulin regulation of lipolysis [11] could increase NEFA, flux to other tissues like skeletal muscle thus increasing their triglyceride storage [12, 13], altering the hepatic glucose output [14] and insulin secretion. Increased fat accumulation can also act in a paracrine and/or endocrine way to promote insulin resistance by thus far unknown mechanisms [15]. In this context composition of the fat could also be important since the increase in saturated FA [4, 5] was associated with insulin resistance. An increase in monounsaturated FAs (MUFA) in serum FA, in kidney and heart phospholipids fractions of obese Zucker rats in comparison to lean littermates has been reported [16]. In humans, MUFA concentrations in muscle phospholipids were positively correlated with fasting plasma insulin concentrations but negatively with muscle content of polyunsaturated

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*Abbreviations:* PL, phospholipids; DG, diglycerides; TG, triglycerides; CE, cholesterol ester; GR, glucose-infused rats; C, control rats; PKC, protein kinase C; SFA, saturated fatty acids; PUFA, sum of all polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; GUFA, all groups of unsaturated fatty acids; SCD, stearoyl-CoA desaturase; GC. gas chromatography.

fatty acid (PUFA) [4]. The altered FA composition in lipid membranes of skeletal muscle in insulin-resistant, obese or diabetic rodents and humans could be caused by an altered FA synthesis pattern. This might be due to altered enzyme activities catalysing the elongation and desaturation process of FA [17] in the liver with subsequent transport to the skeletal muscle and/or due to local change in FA synthesis pattern, although the rates of de novo lipogenesis are believed to be low in skeletal muscle in both man and experimental animals [18].

For the formation of long-chain MUFA and PUFA, FA are desaturated and elongated with the help of Δ5-, Δ6- and Δ9-desaturases which insert a double bond at the fifth, sixth and ninth carbon from the carboxyl terminal, respectively [19, 20]. Elongation is processed by the ubiquitous elongase, which inserts two carbon units at the carboxyl terminal of FA [17].

Numerous reports have focused on the role of high fat diets on insulin action in skeletal muscle [21, 22, 23, 24, 25]. Consistently, it has been reported that high fat diet increases the proportion of saturated fatty acids in the skeletal muscle membranes. Studies in rats with high sucrose diets have shown that the substitution of carbohydrate for fat could also result in increased rat muscle triglyceride content, impaired glucose tolerance [9, 26, 27] and increased long chain fatty acid-CoA accumulation in skeletal muscle [8]. It seems that high sucrose diets might cause insulin resistance only when they result in a positive energy balance leading to more weight gain than in control animals [27]. Although most of these studies indicate that dietary carbohydrates influence fatty acid composition in skeletal muscle and could lead to insulin resistance, the effect of carbohydrate oversupply on the lipid metabolism of the different lipid fractions has not been studied explicitly.

The aim of this study was to investigate the relationship between carbohydrate oversupply, insulin resistance and FA composition in different lipid fractions of skeletal muscle in an animal model as was originally introduced by another study [28].

## Materials and methods

*Materials*. The materials used in this study were purchased as follows (company in brackets): Ketamine (Ketanest, Parke-Davis, Freiburg, Germany), Rompun (Bayer, Leverkusen, Germany) silicone rubber (Silastic, Dow Corning, Midland, Mich., USA), heparin (Liquemine, Roche, Grenzach, Switzerland), swivel (ZAK-Medizintechnik, Munich, Germany), syringe pump (Perfusor, B. Braun, Melsungen, Germany), 50% glucose (Fresenius, Bad Homburg, Germany), Dismembrator S (B. Braun, Melsungen, Germany), [9,10-$^3$H]stearoyl-CoA (Biotrend Chemicals, Cologne, Germany), Norit A (Norit, Düsseldorf, Germany).

*Animals*. All procedures carried out in this study were approved by the local Animal Experimentation Ethics Committee and the "principles of laboratory animal care" (NIH publication no. 85–23, revised 1985) were followed. Female Wistar rats weighing about 300 g were purchased from Charles River, Sulzfeld, Germany and were kept at 22°C with a 12 h light to darkness cycle and a relative humidity of 55 to 60% during the whole experimental period. The rats were given free access to water and standard chow pellet diet (Altromin 1324, Altromin-Futterwerk, Lage, Germany).

*Prolonged glucose infusion into conscious rats*. Glucose infusion was done as previously described [29]. After placing the catheter rats, were allowed to recover for 48 h, after which glucose infusion (2.77 mol/l glucose) was started at a rate of 2 ml/h (GR) compared with 77 mmol/l saline infusion at 2 ml/h (C). Rats were allowed water and chow pellet freely. To measure plasma glucose and insulin concentrations blood samples were taken from the tail vein [29]. After day 2 or day 5 of continuous infusion rats were killed and mixed hind-limb muscles were dissected free of connective tissue and fat, cut into small pieces and subsequently stored at −80°C and subsequently analysed for glycogen and triglycerides [30].

*Skeletal muscle lipid analysis*. Samples of 10 mg skeletal muscle free of visible fat and connective tissue were suspended in 1 ml phosphate-buffered saline, vortexed and further homogenised by ultrasonication. Subsequently lipids were extracted using 2.5 ml of isopropanol:n-heptane:phosphoric acid (40:20:1, vol/vol), vortexed and left to stand for 10 min at room temperature. After centrifugation the supernatant was quantitatively aspirated and completely dried under nitrogen stream. Extracts were resolved in 75 µl chloroform:methanol (2:1) and fractionated by thin-layer chromatography using thin layer plates (Merck, Darmstadt, Germany) coated with 0.25 mm silica gel. Plates were preconditioned by heating at 100° C for 2 h and were developed (20–30 min) using hexan:diethylether:acetic acid (27:7:1 vol/vol). The plates were allowed to dry on air and the separated standard lipid fractions were sprayed with 0.5% 2,7-dichlorofluoresceine in methanol and were visualised under ultraviolet light according to the standards. Five lipid fractions, ie. phospholipids (PL), triglycerides (TG), diglycerides (DG), NEFA and cholesterol ester (CE) were scraped off and lipids were extracted with 2 ml methanol:toluol (1:5). Acetylchlorid (200 µl) was added and agitated for 1 h at 100°C, then cooled extracts were treated with 5 ml 0.43 M $K_2CO3$, mixed for 2 min and centrifuged at 4000 rpm for 10 min. The upper phase was transferred quantitatively to GC-vials and dried down to 80 µl under a nitrogen stream. Fatty acid methyl esters of the separated fractions were analysed by gas-liquid chromatography using an HP 5890 A apparatus (Hewlett Packard, Waldbrunn, Germany) equipped with 60 m×0.25 mm i.d. fused silica column coated with a 0.2 µm film of Rtx 2331 (Restek, Bad Homburg, Germany) and detected by flame ionisation detector.

*Enzyme activity index*. Enzyme activity indices were obtained by relating the amount of the specific substrate to the corresponding product of the respective enzyme [4, 20].

*Preparation of microsomal fractions and assay of Δ9-desaturase activity*. The assay was carried out according to a published procedure [31]. Aliquots of excised muscles were weighed (ca. 200 mg) and ground in a liquid nitrogen-cooled porcelain. Muscles were placed in a liquid nitrogen-cooled Dismembrator S and powdered at a setting of 2000 rpm for 1 min. Powdered muscles were suspended in 1 ml ice-cold buffer containing 10 mmol/l Tris, pH 7.4, 1 mmol/l dithiothre-

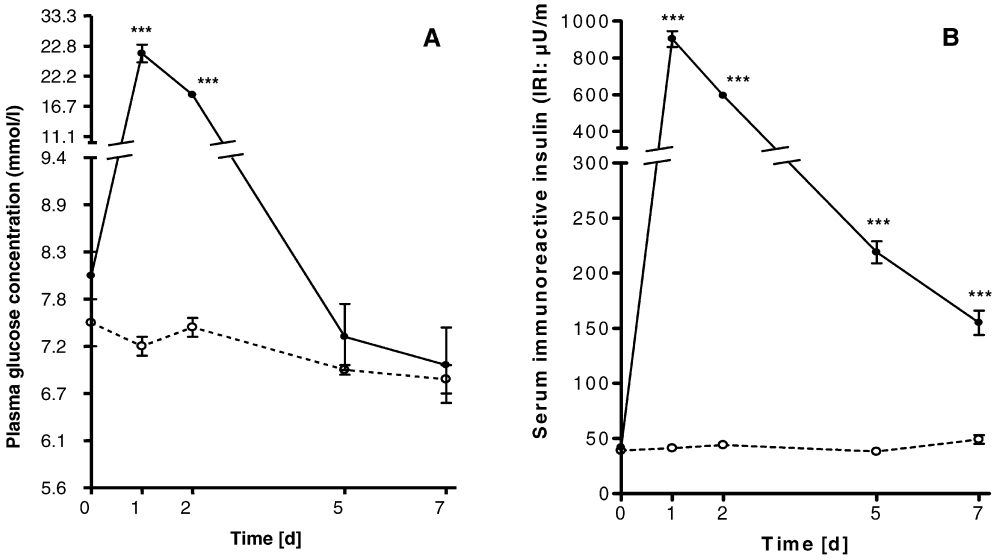


**Fig. 1 (A, B).** Time-dependent effect of glucose infusion on plasma glucose (**A**) and plasma insulin (**B**) concentrations in rats. Continuous glucose infusion (2.77 mol/l) into rats (full circles) was carried out. Control rats received continuous infusion of (77 mmol/l) NaCl (open circles) at the same infusion rate of 2 ml/h. Blood samples were taken from the tail vein and glucose and immunoreactive insulin (IRI) concentrations were measured. Data represent results of five independent experiments; means ± SEM; **p* <0.05, ****p*<0.001

itol and 0.25 mol/l sucrose. Further homogenization was done with a motor-driven Potter-Elvehjem Teflon-glass tissue grinder at a setting 1500 rpm and for approximately ten cycles. Crude muscle homogenate was then spun at 15 000 *g* for 20 min. The supernatant was spun in an ultracentrifuge at 100 000 *g* for 1 h at 4°C using Optima Max centrifuge (Beckman, Munich, Germany). The supernatant was discarded, and the microsomal fraction was resuspended in 200 µl of 0.1 mol/l sodium phosphate buffer, pH 7.4 and all steps were carried out at 4°C. The protein concentration was then measured using the dye-based Bradford assay (Bio-Rad-Kit) and Δ9-desaturase activity was measured in the microsomal fraction (100 µl) by the generation of $^3H_2O$ from the substrate [9,10-$^3$H]stearoyl-CoA [Biotrend Chemicals, Cologne, Germany, specific activity: $2.2\times10^{12}$ TBq /mmol]. Samples were incubated at 37°C for 5 min, terminated by the addition of 1.3 ml of ethanol, and spun at 15 000 *g* for 5 min. Residual substrate was removed by the addition of 40 mg of Norit A, followed by centrifugation as before, and produced $^3H_2O$ was measured in the supernatant by liquid scintillation counting.

*Preparation of total RNA from rat skeletal muscle and RT-PCR.* Isolation of RNA from skeletal muscle, reverse transcription and PCR were carried out [30]. Primer design was made from a gene sequence of Δ9-desaturase, obtained from the Genome-GenBank. β-Globin was used as external standard for quantification and control rats were set at 1 then a comparison was made to the corresponding treated rats (x-fold of controls). PCR product sizes were verified by gel electrophoresis on 2% agarose.

*Statistical analysis*. All data are expressed as means ± SEM. Data were analysed using analysis of variance with repeated measure design. Data on fatty acids composition are expressed in percent of the corresponding fraction. A *p* value of less than 0.05 was considered to be statistically significant.

## Results

*Metabolic effects of continuous glucose infusion*. Continuous glucose infusion into rats for 7 days induced transient hyperglycaemia and persistent hyperinsulinaemia. Hyperglycaemia peaked after 24 h of glucose infusion (Fig. 1A), and then fell continuously reaching normal values after 5 days (day 5: 7.3±0.4 GR vs 6.9±0.06 mmol/l C) and remained normal after 7 days despite further continuous glucose infusion. Serum insulin concentrations followed a similar pattern (Fig. 1B) but hyperinsulinaemia persisted in GR throughout the glucose infusion period (day 5:219± 10*** GR vs 38±1 µU/ml C). Saline infusion affected neither plasma glucose nor insulin concentration in control rats. Glucosuria was detected in day 2 of the infusion period and a faster weight gain and lower food consumption was seen in glucose-infused rats (Table 1). However, the daily amount of infused glucose was much more than the decreased food consumption. Of note, the hyperglycaemic/hyperinsulinaemic state in day 2 changed to a normoglycaemic/hyperinsulinaemic state in day 5. This resulted in a 15-fold increase in muscle glycogen content after 2 days, which fell to a 3.5-fold increase at day 5 of glucose infusion, whereas triglyceride content remained high.

*Fatty acid composition in the phospholipid fraction of skeletal muscle*. Prolonged glucose infusion did not affect the composition of saturated fatty acids, whereas MUFA increased after day 5 of glucose infusion. Particularly, palmitoleic acid (16:1 N7) increased 2.4-fold and six-fold compared to control values after 2 and 5 days of glucose infusion, respectively. No changes were seen in the composition of PUFAs with

**Table 1.** Effects of continuous glucose infusion on body weight, glucosuria and food consumption in rats and on glycogen and triglyceride content of rat skeletal muscle. Rats ($n$=5) were continuously infused with 2 ml/h glucose solution (2.77 mol/l) for 2 or 5 days (GR). Control rats received 2 ml/h of 77 mmol/l NaCl (C). Data represent means ± SEM; *$p$<0.05, ***$p$<0.001

| Infusion period | Day 2 | | Day 5 | |
|---|---|---|---|---|
| | C | GR | C | GR |
| Glucose infused (mmol/d) | 0 | 133 | 0 | 133 |
| Urine glucose | Negative | Positive | Negative | Negative |
| Food consumption (g/d) | 10±0.3 | 7.8±0.2* | 12±0.3 | 7±0.3* |
| gained weight (g/d) | 1.8±0.1 | 3±0* | 1.8±0.1 | 3.4±0.2* |
| Glycogen content (mg/g muscle) | 3.3±0.7 | 49±5.6*** | 4.5±0.9 | 15.9±2* |
| Triglyceride content (μmol/g muscle) | 244.9±36 | 417±42* | 166.3±29 | 426±74* |

**Table 2.** Effects of continuous glucose infusion on fatty acid composition of phospholipid fraction in rat skeletal muscle. Rats ($n$=5) were continuously infused with 2 ml/h glucose solution (2.77 mol/l) for 2 or 5 days (GR). Control rats received 2 ml/h of 77 mmol/l NaCl (C). Lipids were extracted from skeletal muscle and separated by TLC. The fatty acid composition of the different lipid was analysed by gas chromatography. Phospholipid fraction is set at 100% and individual FA are presented in percent of total phospholipid fraction (means ± SEM); * $p$<0.05. Important changes are indicated in bold

| Glucose Infusion<br>Phospholipids<br>Fatty acids | day 2<br>Composition (%) | | day 5<br>Composition (%) | |
|---|---|---|---|---|
| | C | GR | C | GR |
| 14:0 | 1.37±0.23 | 1.25±0.36 | 1.59±0.46 | 1.20±0.12 |
| 15:0 | 0.75±0.14 | 0.71±0.27 | 0.75±0.14 | 0.58±0.10 |
| 16:0 | 33.6±1.2 | 32.9±1.09 | 34.4±0.83 | 34.1±0.99 |
| 18:0 | 16.3±1.1 | 16.3±0.47 | 16.9±0.92 | 15.7±0.87 |
| 20:0 | 0.19±0.03 | 0.15±0.00 | 0.17±0.03 | 0.16±0.03 |
| 22:0 | 0.26±0.06 | 0.15±0.01 | 0.16±0.03 | 0.22±0.07 |
| **∑SFA** | 52.6±1.08 | 51.4±1.52 | 54.0±2.22 | 51.9±1.26 |
| 16:1 N7 | **0.60±0.09** | **1.44±0.05*** | **0.33±0.03** | **1.99±0.28*** |
| 18:1 N9 | 5.26±0.61 | 4.50±0.22 | **4.24±0.31** | **7.08±0.85*** |
| 18:1 N7 | 1.99±0.09 | 1.99±0.18 | 2.03±0.16 | 2.40±0.10 |
| **∑MUFA** | 7.86±0.60 | 7.93±0.41 | **6.59±0.26** | **11.46±1.11*** |
| 18:2 N6 | 10.38±0.7 | 10.53±0.91 | 10.71±0.3 | 10.53±0.90 |
| 18:3 N6 | 0.06±0.00 | 0.06±0.00 | 0.06±0.01 | 0.07±0.00 |
| 20:3 N6 | 0.34±0.03 | 0.36±0.02 | 0.34±0.03 | 0.38±0.03 |
| 20:4 N6 | 11.96±0.7 | 12.29±0.87 | 12.47±0.95 | 11.57±0.58 |
| 22:4 N6 | 0.12±0.13 | 0.12±0.05 | 0.15±0.04 | 0.15±0.01 |
| **∑N6 PUFA** | 23.31±1.27 | 25.11±0.61 | 24.14±1.2 | 23.06±1.14 |
| 18:3 N3 | **0.34±0.05** | **0.27±0.02*** | **0.37±0.02** | **0.19±0.02*** |
| 20:5 N3 | 0.17±0.03 | 0.12±0.05 | 0.15±0.04 | 0.15±0.01 |
| 22:5 N3 | 1.03±0.10 | 1.09±0.12 | 1.17±0.11 | 1.00±0.08 |
| 22:6 N3 | 14.40±1.22 | 13.71±1.65 | 13.20±1.25 | 11.78±0.58 |
| **∑N3 PUFA** | 15.77±1.26 | 15.07±1.73 | 14.74±1.3 | 12.97±0.66 |
| **GUFA** | 39.58±1.5 | 40.66±1.59 | 39.36±2.4 | 36.57±1.77 |
| N6/N3=PUFA6/3 | 1.52±0.18 | 1.52±0.27 | 1.65±0.08 | 1.77±0.05 |
| (18:0/16:0)x100 (elongase) | 49±4 | 41±9 | 49±2 | 46±3 |
| 16:1 N7/16:0 x100 (Δ9-desaturase) | **2±0.3** | **4±0.1*** | **1±0.1** | **6±1*** |
| (20:4 N6/20:3 N6) (Δ 5 desaturase) | 35±2 | 34±2 | 37±3 | 31±3 |
| 20:3 N6/18:2 N6 (Δ6 desaturase) | 33±1 | 35±3 | 32±3 | 37±2 |

the exception of the ω3 fatty acid α-linolenic acid (18:3 N3) which was reduced in skeletal muscle phospholipids by 50% after 5 days of glucose infusion (Table 2).

To assess changes in enzyme activities of FA metabolism indices relating substrate and product of the respective enzyme reactions were calculated. The index of Δ9-desaturase (measure of activity) was increased two-fold and six-fold after day 2 and day 5 of glucose infusion, respectively, whereas no changes were seen in the activities of Δ5- and Δ6-desaturases nor in that of elongase.

**Table 3.** Effects of continuous glucose infusion on fatty acid composition of triglycerides fraction. Rats ($n$=5) were continuously infused with 2 ml/h glucose solution (2.77 mol/l) and fatty acid content in the lipid fraction was analysed. Triglyceride fraction is set at 100% and individual FA are shown in percent of total triglyceride fraction (means ± SEM). Important changes in fatty acids in the treated group compared with the control group are marked with * ($p$<0.05) and indicated in bold

| Glucose Infusion<br>Triglycerides<br>Fatty acids | day 2<br>Composition (%) | | day 5<br>Composition (%) | |
|---|---|---|---|---|
| | C | GR | C | GR |
| 14:0 | 2.52±0.12 | 2.51±0.45 | 2.82±0.85 | 2.10±0.42 |
| 15:0 | 0.34±0.07 | 0.68±0.29 | 0.59±0.17 | 0.32±0.03 |
| 16:0 | 31.74±1.1 | 33.03±1.4 | 31.15±2.6 | 33.7±0.8 |
| 18:0 | 5.5±0.13 | 5.4±0.32 | **7.27±1.5** | **3.78±0.04*** |
| 20:0 | 0.11±0.02 | 0.10±0.01 | 0.14±0.03 | 0.07±0.01 |
| 22:0 | 0.06±0.01 | 0.08±0.01 | 0.07±0.00 | 0.07±0.01 |
| **∑SFA** | 40.5±1.1 | 41.8±2.43 | 42.05±5.08 | 40±1.1 |
| 16:1 N7 | **6.99±0.48** | **8.08±0.7*** | **5.28±1.4** | **13.7±0.9*** |
| 18:1 N9 | 23.53±0.9 | 21.67±1 | **20.67±0.8** | **23.55±0.3*** |
| 18:1 N7 | 2.48±0.12 | 2.4±0.13 | 2.54±0.15 | 2.6±0.13 |
| **∑MUFA** | 32.99±0.93 | 32.14±1.6 | **28.5±1.5** | **39.83±0.9*** |
| 18:2 N6 | 23.07±1.4 | 22.4±0.83 | **25.6±4** | **17.33±1.5*** |
| 18:3 N6 | 0.07±0.01 | 0.06±0.01 | 0.07±0.01 | 0.06±0.01 |
| 20:3 N6 | 0.09±0.02 | 0.10±0.01 | **0.15±0.02** | **0.08±0.01*** |
| 20:4 N6 | 0.8±0.15 | 0.93±0.2 | 1.0±02 | 0.7±0.1 |
| 22:4 N6 | 0.61±0.27 | 0.65±0.25 | 0.55±0.12 | 0.44±0.12 |
| **∑N6 PUFA** | 24.54±1.7 | 24.00±1.05 | 27.2±4.2 | 18.52±1.7 |
| 18:3 N3 | 1.5±0.25 | 1.5±0.21 | 1.6±0.4 | 1.2±0.23 |
| 20:5 N3 | 0.04±0.01 | 0.03±0.01 | 0.06±0.01 | 0.03±0.01 |
| 22:5 N3 | 0.1±0.03 | 0.12±0.03 | 0.13±0.03 | 0.09±0.02 |
| 22:6 N3 | 0.23±0.08 | 0.4±0.08 | 0.34±0.05 | 0.25±0.05 |
| **∑N3 PUFA** | 1.86±0.3 | 1.97±0.31 | 2.11±0.5 | 1.54±0.3 |
| **GUFA** | 26.5±1.9 | 26.1±1.34 | 29.5±4.7 | 20.2±1.95 |
| N6/N3=PUFA6/3 | 12.86±1.95 | 11.91±1.05 | 13.06±0.84 | 13.02±1.6 |
| (18:0/16:0)x100 (elongase) | 17±1 | 16±8 | **23±3** | **11±0.3*** |
| (16:1 N7/16:0)x100 (Δ9-desaturase) | 22±1 | 25±3 | **17±4** | **41±2*** |
| 20:4 N6/20:3 N6 (Δ5 desaturase) | 8±0.7 | 9±0.5 | 8±1 | 9±0.4 |
| 20:3 N6/18:2 N6 (Δ6 desaturase) | 4±0.5 | 5±0.4 | 5±0.4 | 5±0.2 |

The index of Δ9-desaturase was increased 2.4-fold whereas that of elongase decreased by about 50%. No significant changes were seen in Δ5- and Δ6-desaturase activity (Table 3).

*Fatty acid composition in the diglycerides fraction.* With the exception of stearic acid (18:0) and arachidonic acid (20:0), which were decreased in the muscle of GR at day 5, prolonged glucose infusion did neither affect the composition nor the absolute content of saturated fatty acids, whereas MUFAs composition increased (~1.3-fold) after day 2 (GR 20.75±1.16 vs C 15.9±0.92) and after day 5 (~1.9-fold) (GR 22.46± 1.4 vs C 11.88±1.6) of glucose infusion when compared to control rats. The increased composition of MUFA was again due to an increased relative content of palmitoleic acid (16:1 N7) after day 2 (1.9-fold) (GR 5.03±0.33 vs C 2.67±0.37) and day 5 (4.1-fold) (GR 6.6±0.67 vs C 1.6±0.67) of glucose infusion. No changes were seen in the composition of PUFAs.

The index of Δ9-desaturase was increased 2.1-fold after day 2 (GR 15±2 vs C 7±1) and 4.3-fold after day 5 of glucose infusion (GR 17±2 vs C 4±2). No changes were seen in the activities of Δ5- and Δ6-desaturases, except for Δ5-desaturase after day 5 (1.6-fold increase) (GR 11±1 vs C 7±1), while elongase activity index decreased by 25% after day 5 of glucose infusion (GR 45±2 vs C 60±3).

*Fatty acid composition in the free fatty acid fraction.* Prolonged glucose infusion did neither affect the composition nor the absolute content of saturated fatty acids, whereas MUFAs composition increased after day 2 (1.3-fold) (GR 16.87±2.46 vs C 12.7±1.2) and after day 5 (1.6-fold) of glucose infusion when compared to control rats (GR 18.3±1.1 vs C 11.34±0.88). Again, the increased composition of MUFA was essentially due to increased palmitoleic acid (16:1 N7) content (2.5-fold after day 2 and 2.5-fold after day 5) (2 day: GR 5±0.3 vs C 2±0.4; 5 day: GR 5±0.6 vs C 2±0.4) whereas no changes were seen for PUFAs.

*Fatty acid composition in the triglycerides fraction.* With exception of stearic acid (18:0), which was relatively decreased in GR muscle after day 5, prolonged glucose infusion did neither affect the composition nor

**Table 4.** Indices of enzyme activities of elongase and different desaturases (desat.) Effects of continuous glucose infusion on FA metabolising enzyme activities in rat skeletal muscle. Enzyme activity indices were calculated by forming the ratio of the corresponding product/substrate using results shown in tables 2 and 3 and in the text. Activities are expressed as x-fold increase relating to control animals,*n*=5. Important changes are indicated in bold

| | Elongase | | Δ9-desat. | | Δ5-desat. | | Δ6-desat. | |
|---|---|---|---|---|---|---|---|---|
| | day 2 | day 5 | day 2 | day 5 | day 2 | day 5 | day 2 | day 5 |
| PL | n.s. | n.s. | **2** | **6** | n.s. | n.s. | n.s. | n.s. |
| TG | n.s. | **0.5** | n.s. | **2.4** | n.s. | n.s. | n.s. | n.s. |
| DG | n.s. | **0.7** | **2.1** | **4.3** | n.s. | **1.6** | n.s. | n.s. |
| NEFA | n.s. | n.s. | **2.3** | **3.5** | **1.6** | n.s. | n.s. | n.s. |

PL=phospholipid, TG=triglyceride, DG=diglyceride, NEFA

the absolute content (not shown) of saturated fatty acids. The composition of MUFAs was unchanged after day 2 of glucose infusion when compared to control rat, whereas an increase was seen in GR muscle after 5 days of glucose infusion (~1.4-fold of control). The increase was particularly obvious for palmitoleic acid (16:1 N7) (2.6-fold). No changes were seen in the composition of PUFAs with exception of the ω6 fatty acids (18:2 N6) and (20:3 N6), which were decreased in GR after 5 days of glucose infusion (Table 3).

In parallel, the index of Δ9-desaturase was increased 2.3-fold and 3.5-fold after day 2 and day 5 of glucose infusion, respectively (2 day: GR 14±1 vs C 6±0.8; 5 day: GR 14±2 vs C 4±1). No changes were seen in the activity indices of Δ6-desaturase and elongase whereas the index of Δ5-desaturase was increased by 1.6-fold after day 2 (GR 18±2 vs C 11±1).

In the cholesterol ester fraction, which was very low in rat skeletal muscle (<10% of triglyceride content), we found no significant changes in any of the fatty acids measured (data not shown).

*Enzyme activity indices of the fatty acid metabolism.* The determination of the composition of the lipid fraction of skeletal muscles showed that glucose infusion specifically influenced the content of different fatty acids, however, palmitoleic acid (16:1 N7) was increased in all lipid fractions. From the summarised activity indices of the enzymes necessary for the processing of fatty acids (Table 4) it seems that glucose oversupply did not affect Δ5- and Δ6-desaturases in most lipid fractions studied (in two fractions <two-fold) while the activity indices for Δ9-desaturase were increased more than two-fold after day 2 (in three out of four lipid fractions) and 2.4-fold to six-fold (in all four lipid fractions) after day 5 of glucose infusion. These data indicate that glucose oversupply specifically enhances Δ9-desaturase activity.

*Effect of continuous glucose infusion on Δ9-desaturase mRNA expression and enzyme activity in rat skeletal muscle.* A rapid turnover rate has been recently shown for the microsomal enzyme Δ9-desaturase [32].

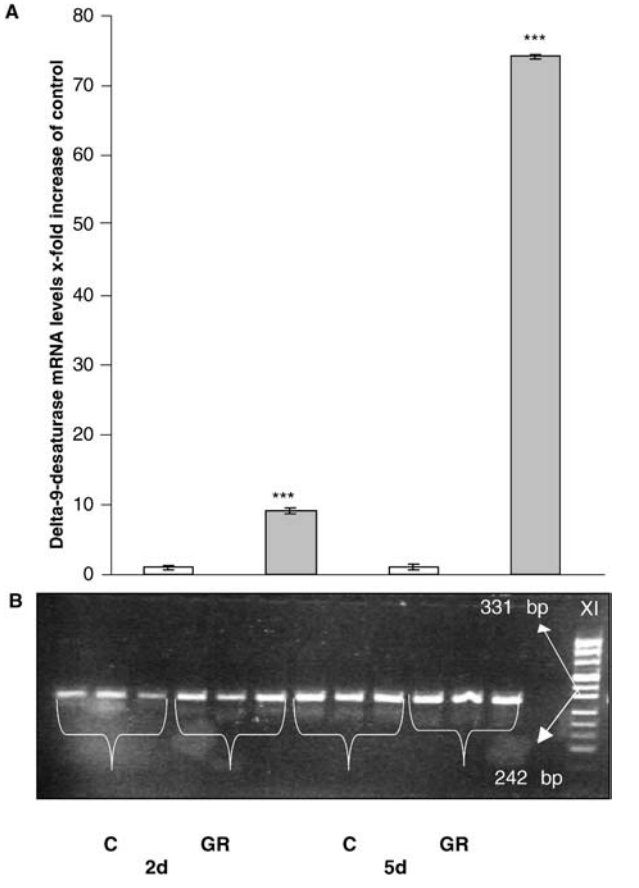


**Fig. 2A, B.** Time-dependent effect of glucose infusion on Δ9-desaturase mRNA contents in rat skeletal muscle. (**A**) RNA was extracted from skeletal muscle of control (open bars) and glucose-infused (filled bars) rats and Δ9-desaturase mRNA were measured by RT-PCR using real time. Controls were set at 1 and GR were shown as x-fold of increase of controls. Data represent results of five independent experiments; means ± SEM; ***$p$<0.001. (**B**) Representative post run agarose electrophoresis showing the size of Δ9-desaturase PCR-product. RT-PCR was monitored in real-time using the Light Cycler online monitoring. Post run PCR-products were collected from PCR capillaries and loaded on 2% agarose gel. Electrophoresis was run, gel was placed on UV-transilluminator and photographed using Medidoc gel documentation system (Herolab, Wiesloch, Germany)

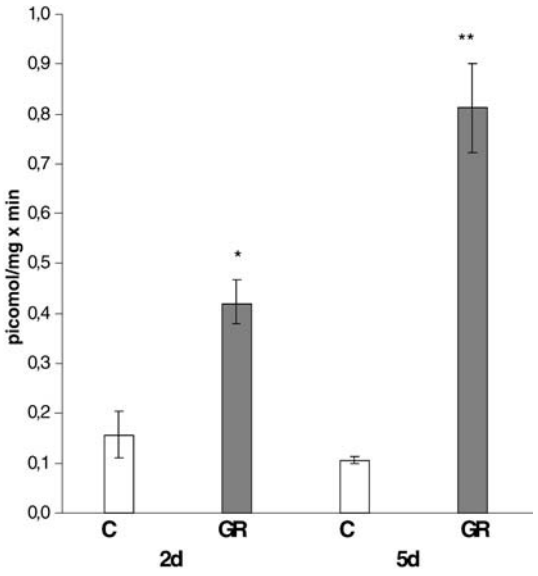


**Fig. 3.** Time-dependent effect of glucose infusion on Δ9-desaturase enzyme activity in rat skeletal muscle. Protein was extracted from skeletal muscle of control (open bars) and glucose-infused (filled bars) rats and specific activity of Δ9-desaturase was measured in the microsomal. Data represent results of three independent experiments; means ± SEM; $^{**}p<0.01$; $^{*}p<0.05$

To assess if the effect of glucose oversupply on MUFA is caused by an increase in Δ9-desaturase turnover, the content of Δ9-desaturase mRNA was measured in rat skeletal muscle by real time RT-PCR. As shown in Fig. 2A we observed a 10-fold and 75-fold increase in Δ9-desaturase mRNA content after day 2 and day 5 in skeletal muscle of glucose infused rats, respectively. The size of the PCR products were evaluated by agarose electrophoresis (Fig. 2B). All PCR products showed the expected size after finishing real time PCR. These data indicate that glucose oversupply induces the expression of Δ9-desaturase mRNA.

To further verify the specific induction of Δ9-desaturase in skeletal muscle, enzyme activity studies were carried out as described. In concomitance to the calculated activity indices Δ9-desaturase enzyme activities were increased 2.6-fold and 7.7-fold after 2 and 5 days of glucose infusion, respectively (Fig. 3).

## Discussion

This study was designed to investigate the relationship between glucose oversupply, insulin resistance and lipid composition in rat skeletal muscle. The results describe the effects of 2 and 5 days of glucose infusion, a condition previously described to cause insulin resistance in rat skeletal muscle, on the composition of FA in different lipid fractions. The main findings of this study are that prolonged glucose infusion induced (i) specific changes in the composition of the fatty acids in phospholipids, NEFA, triglycerides and diglycerides, ie. the relative content of palmitoleic acid (16:1 N7) increased several-fold in all lipid fractions (ii) a striking increase in Δ9-desaturase mRNA expression and concomitant but lower increase in enzyme activity after day 2 and day 5. Furthermore, glucose oversupply led to a decrease in the relative content of some PUFA; in fact, GUFA were decreased in nearly all lipid fractions, however, in most cases the reduction failed to reach statistical significance. The measured increase in total triglyceride content confirms earlier results obtained with this animal model [8].

Our finding that increasing Δ9-desaturase mRNA concentrations are higher than the corresponding Δ9-desaturase enzyme activities are well in line with previous results [32, 33]. The differences could be explained by the fact that the half-life of Δ9-desaturase is very short [32] thus rapid changes of mRNA levels are not equivalently translated into a corresponding increase in Δ9-desaturase enzyme activity. It is important to note that Δ9-desaturase activity estimated in microsomes isolated from frozen tissues is lower than in fresh tissue.

While glucose oversupply obviously increased palmitoleic acid content in the intermediates of the lipid metabolism, ie. NEFA and diacylglycerides, the effect on triglycerides, the storage lipid, was lower after 2 days but more pronounced after 5 days. The glucose oversupply also affected the composition of phospholipids, which play an important functional role in membranes. Changes in the FA composition of phospholipids might alter membrane fluidity and permeability as was shown by the "leaky membrane" hypothesis [34], and probably diminishes insulin sensitivity due to altered insulin receptor number, reduced insulin binding capacity and/or altered insulin receptor tyrosine kinase activity [35, 36, 37, 38] or even more due to altered post receptor signalling [39, 40, 41, 42]. Using the same samples as in this study we found, that in rat skeletal muscle early steps of insulin signalling (phosphorylation of the insulin receptor, IRS-1 and protein kinase B and the IRS-1-associated phosphatidyl-inositol-3′-kinase activity) are inhibited after 2 and particularly after 5 days of glucose infusion[30]. Activation of PKC has been implicated in the development of insulin resistance [8, 43, 44, 45, 46]. Of note, we found an activation of PKC, particularly the isoform β, in skeletal muscle of glucose infused rats [30]. Whether an activation of PKC could be related to the altered FA composition in GR and to the Δ9-desaturase induction remains to be shown.

Our data propose some links between glucose oversupply induced insulin resistance and specific changes in the FA composition of skeletal muscle lipids. The findings of a fast and specific increase of palmitoleic and oleic acid together with an enhanced expression of Δ9-desaturase indicate that skeletal muscle contains, in addition to the previously shown rapidly reg-

ulated lipolysis [38], a rapidly and specifically regulated FA metabolism. Our results support the suggestion that altered FA composition in lipid membranes of skeletal muscle in insulin resistant, obese or diabetic rodents and humans might be due to different desaturation availability of the different FA rather than altered fatty acid uptake [13] since different fatty acids seem not to compete for tissue entry or esterification [47].

Recent reports show that the gene expression of Δ9-desaturase is highly regulated (reviewed in [48]). The finding that the half-life time of Δ9-desaturase is very short indicates that Δ9-desaturase may be a key regulatory enzyme in lipid metabolism [32]. High carbohydrate and insulin induce the hepatic expression of Δ9-desaturase [32, 48]. In rat liver microsomes Δ9-desaturase can be induced more than 50-fold by the administration of a fat-free high-carbohydrate diet. Abrupt termination of the dietary regimen causes rapid decrease of the Δ9-desaturase activity and the protein content to very low amounts [49]. Little seems to be known about the regulation of Δ9-desaturase in skeletal muscle, the main target tissue of insulin-stimulated glucose uptake. In particular, it has been shown that Δ9-desaturase activity is increased in skeletal muscle of obese Pima Indians and that this increase is independently correlated with insulin sensitivity and obesity in these subjects [50]. We report on the in vivo regulation of Δ9-desaturase in insulin-resistant skeletal muscle of GR, ie. increased MUFAs and increased Δ9-desaturase activity index in nearly all lipid fractions of GR. Our findings that Δ9-desaturase mRNA expression and enzyme activities were substantially increased after 2 and 5 days in skeletal muscle argues for a local desaturation process independent of a possible enhanced synthesis of MUFA in liver with subsequent transport to muscle. Of note, we observed important Δ9-desaturase mRNA contents and Δ9-desaturase activities in myotubes obtained from human skeletal muscles supporting our findings (E. Schleicher, unpublished observation). Our results suggest a correlation of high Δ9-desaturase activity with obesity, muscle insulin resistance and possibly diabetes. Our data add to the recent discovery of the key role of Δ9-desaturase in metabolism and energy balance [51].

Previous reports have indicated that elongase activity (18:0/16:0) is reduced in insulin resistance [16, 50, 52, 53]. In this study, we found that elongase activity was decreased in day 5 as assessed by the activity indices found in triglycerides and diglycerides fractions. These data are in accordance with recent reports showing decreased elongase activity (increased C16:0 on the costs of C18:0) in muscle of healthy subjects made insulin resistant [7]. In obesity and insulin resistance states, Δ5-desaturase activity was reported to be reduced [4, 16, 52, 54], whereas that of Δ6-desaturase increased [16]. We found little change in Δ5-desaturase and no change in Δ6-desaturase activity indices. In accordance to our findings no changes in the activities of Δ5- and Δ6-desaturases were found when insulin resistance was induced in normal subjects [7].

In conclusion, continuous glucose infusion into rats, which causes insulin resistance, leads to an increased accumulation of MUFA in membrane phospholipids and in the storage lipid triglyceride in skeletal muscle. The increases in MUFA could be caused by the strikingly increased Δ9-desaturase activity. Our results indicate a key regulatory role of this enzyme in lipid metabolism of skeletal muscle in insulin resistant states. Although a causal role of Δ9-desaturase in insulin resistance remains to be shown, increased concentrations of palmitoleic acid (16:1N7) may serve as a marker in insulin resistance of skeletal muscle.

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