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Short-term increase of plasma free fatty acids does not interfere with intrinsic mitochondrial function in healthy young men

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ABSTRACT

Free fatty acid (FFA)– and obesity-induced insulin resistance has been associated with disturbed mitochondrial function. Elevated plasma FFA can impair insulin-induced increase of adenosine triphosphate synthesis and downregulate the expression of genes important in the biogenesis of mitochondria in human skeletal muscle. Whether FAs have a direct effect on intrinsic mitochondrial capacity remains to be established. Therefore, we measured ex vivo mitochondrial respiratory capacity in human skeletal muscle after exposure to hyperinsulinemia and high levels of plasma FFA. Nine healthy lean men were studied during a 6-hour hyperinsulinemic (600 pmol/L) euglycemic clamp with concomitant infusion of Intralipid (Fresensius Kabi Nederland, Den Bosch, the Netherlands) (FFA clamped at 0.5 mmol/L) or saline. Mitochondrial respiratory capacity was measured by high-resolution respirometry in permeabilized muscle fibers using an Oxygraph (OROBOROS Instruments, Innsbruck, Austria). Each participant served as his own control. Peripheral glucose uptake (rate of disappearance) was significantly lower during infusion of the lipid emulsion compared with the control saline infusion (68 μmol/kg·min [saline] vs 40 μmol/kg·min [lipid], $P = .008$). However, adenosine diphosphate–stimulated and maximal carbonylcyanide-4-(trifluoromethoxy)-phenylhydrazone–stimulated uncoupled respiration rates were not different in permeabilized skeletal muscle fibers after exposure to high levels of FFA compared with the control condition. We conclude that short-term elevation of FFA within the physiological range induces insulin resistance but does not affect intrinsic mitochondrial capacity in skeletal muscle in humans.

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1. Introduction

The prevalence of diabetes mellitus type 2 (DM2) is rapidly increasing. This increment runs in parallel with the pandemic increase in the prevalence of obesity. Obese insulin-resistant subjects as well as patients with DM2 have elevated plasma free fatty acid (FFA) levels [1]. The increased levels of FFA may directly mediate the development of insulin resistance [2,3]. Indeed, experimental elevation of plasma fatty acids in lean healthy subjects by infusion of a lipid emulsion reduces hepatic and peripheral insulin sensitivity by similar metabolic disturbances as described in obese patients with DM2, that is, increased concentrations of intracellular fatty acid metabolites and impaired insulin signaling [4-7]. However, the exact underlying mechanism of FFA-induced insulin resistance has not been fully elucidated.

Studies in obese DM2 subjects revealed increased intracellular accumulation of FA metabolites and impaired mitochondrial function [8]. It has been hypothesized that increased lipid availability could reduce mitochondrial oxidative capacity and exacerbate lipid storage in skeletal muscle, leading to lipotoxicity and insulin resistance [9]. Increased lipid availability could have a deleterious effect on mitochondrial function via (1) downregulation of the expression of genes involved in the biogenesis of mitochondria [10,11], (2) mitochondrial damage by peroxidation of fatty acids [12,13], and (3) incomplete β-oxidation leading to intramitochondrial accumulation of acyl–coenzyme A [14]. Mitochondrial damage by peroxidation of fatty acids will cause an intrinsic mitochondrial defect as it deteriorates the function of the respiratory chain. Accumulation of acyl–coenzyme A may cause a functional impairment and may be reversible, as it has been shown that, in vitro, the addition of FFA to isolated mitochondria decreases mitochondrial respiration, but that this effect is quickly reversed when lipids are washed away [15].

In combination with hyperinsulinemia, short-term elevation of plasma FFA inhibited insulin-stimulated adenosine triphosphate (ATP) production in vivo [16]. This effect was only seen after 6 hours of exposure to high plasma FFA during hyperinsulinemia, whereas 3 hours of exposure had no effect on ATP production [17]. It was therefore suggested that reduced mitochondrial ATP production may be secondary to insulin resistance or energy demand and substrate availability and not caused by an intrinsic mitochondrial defect induced by FFA.

Ex vivo mitochondrial respirometry enables the measurement of mitochondrial capacity under similar substrate conditions. Therefore, to study whether *intrinsic* mitochondrial capacity is affected by high levels of FFA, we measured in lean subjects ex vivo mitochondrial respiration in human skeletal muscle after a 6-hour period of high vs low circulating FFA, while assessing insulin sensitivity by a hyperinsulinemic euglycemic clamp.

2. Methods and procedures

2.1. Experimental subjects

Nine healthy lean white men with a median age of 24 (19-30) years and body mass index of 21.4 (19.6-25.7 kg/m^2) were included in this study. Participants had no family history of DM2 and had a normal glucose tolerance, assessed by an oral glucose tolerance test interpreted according to the American Diabetes Association criteria [18].

Each participant served as his own control. Participants were instructed not to change their physical activity or diet during the study. To achieve homogeneity of the study group, we also checked the participants on physical fitness (participants were not included if their maximum oxygen consumption [Vo_{2max}] was <45 mL/kg·min or >60 mL/kg·min).

The protocol was approved by the Medical Ethical Committee of the Academic Medical Center in Amsterdam. All participants signed a written informed consent before inclusion.

2.2. Vo_{2max} testing

For the assessment of exercise capacity, we performed symptom limited cardiopulmonary exercise test according to the guidelines of the American Thoracic Society [19]. Subjects were placed on a cycle ergometer in the upright position, and continuous measurements were made of minute ventilation, oxygen consumption (Vo_2), carbon dioxide production (Vco_2), heart rate, blood pressure, and electrocardiography (Jaeger Oxycon pro, Wurzburg, Germany). Work load was increased by 15 to 25 W, depending on the predicted maximum exercise capacity, in such a way that maximal effort was attained within 10 to 15 minutes. Calibration of the system occurred before every test according to specifications of the manufacturer.

2.3. Study design

Participants were studied in balanced assignment on 2 different occasions (at least 2 weeks apart) during a 6-hour intravenous infusion of Intralipid (Fresensius Kabi Nederland, Den Bosch, the Netherlands) or control saline 0.9%.

Participants were admitted to the metabolic unit at 7.30 AM after an overnight fast. They were instructed not to exercise or drink alcohol, and to consume 250 g of carbohydrates per day during the 3 days before each study day.

After weight and body composition measurement using bioelectrical impedance analysis, a catheter was inserted into the dorsal vein of each hand. One catheter was used for infusion of insulin, glucose saline, or the lipid emulsion, and the stable glucose isotope. The other catheter was kept in a thermoregulated (60°C) Plexiglas box for sampling of arterialized venous blood. To keep the sampling line patent, a slow infusion of NaCl 0.9% was used.

At T = 0 (8:00 AM), a blood sample was drawn for determination of [6,6-$^2H_2$]glucose background enrichment; and a primed continuous infusion of [6,6-$^2H_2$]glucose was started: prime, 8.8 μmol/kg; continuous, 0.11 μmol/kg·min. At T = 1 hour (9:00 AM), blood samples were drawn for basal measurements of plasma glucose, free fatty acids, and insulin. Thereafter, the infusion of insulin (60 mU/m^2·min) (Actrapid 100 IU/mL; Novo Nordisk Farma, Alphen aan den Rijn, the Netherlands) was started. Plasma glucose concentration was measured every 5 minutes, and a 20% glucose solution was infused at a variable rate to maintain euglycemia at 5 mmol/L.

The 20% glucose solution was enriched with [6,6-$^2H_2$]glucose to approximate the values for enrichment reached in plasma, thereby minimizing changes in isotopic enrichment due to changes in the infusion rate of exogenous glucose. At the start of the clamp, a concomitant infusion of Intralipid 20% or control saline was started for 6 hours at a rate of 100 mL/h.

The plasma FFA concentration was measured every 30 minutes, and the lipid infusion rate was adjusted to clamp the FFA levels at approximately 0.5 mmol/L. The control saline infusion was kept at 100 mL/h.

At the end of the clamp, T = 7 hours (3:00 PM), 5 blood samples were drawn at 5-minute intervals for determination of glucose enrichment, insulin, and FFA concentration.

2.4. Muscle biopsies

After the last blood sample was drawn (ie, in the insulin-stimulated state after 6 hours of hyperinsulinemia), a muscle biopsy was taken from the musculus vastus lateralis under local anesthesia with Lidocaine 20% (Fresenius Kabi; Den Bosch, the Netherlands). Biopsies were performed using an automatic biopsy instrument (Pro-Mag I 2.5; Medical Device Technologies, Gainesville, FL). One part of the biopsy was washed with 0.9% NaCl fortified with 10 mmol/L Na-Hepes to reduce blood contamination, thereafter snap-frozen in liquid nitrogen, and stored at –80°C. The other part was collected in a test tube on ice with 10 mL of ice cold BIOPS solution containing 10 mmol/L Ca-EGTA buffer, 0.1 μmol/L free calcium, 20 mmol/L imidazole, 20 mmol/L taurine, 50 mmol/L K-Mes, 0.5 mmol/L DTT, 6.65 mmol/L $MgCl_2$, 5.77 mmol/L ATP, and 15 mmol/L phosphocreatinine (pH 7.1) and used to measure mitochondrial respiration after permeabilization (see below).

2.5. Body composition

Body composition was measured with bioimpedance analysis (Maltron BF-906; Maltron International, Essex, England).

2.6. Indirect calorimetry

The V_{O_2} and V_{CO_2} were measured during the final 20 minutes of the clamp using a ventilated hood system (Vmax model 2900; SensorMedics, Anaheim, CA).

The V_{O_2} and V_{CO_2} measurements during the last 10 minutes were used for the calculation of the respiratory exchange ratio and rates of fatty acid oxidation and glucose oxidation [20].

2.7. Glucose, insulin, and FFA concentrations

Glucose concentrations were measured using a Biosen C-line plus glucose analyzer (EKF Diagnostics, Barleben/Magdeburg, Germany). [6,6-$^2H_2$]glucose enrichment was measured as described before [21].

Insulin was determined on an Immulite 2000 system (Diagnostic Products, Los Angeles, CA). Insulin was determined with a chemiluminescent immunometric assay. Intraassay variation was 3% to 6%, total assay variation was 4-6%, and detection limit was 15 pmol/L.

Plasma FFAs were measured by an enzymatic method (Nefac; Wako Chemicals, Richmond, VA.) Intraassay variation was 1%, total assay variation was 4% to 15%, and detection limit was 0.02 mmol/L. Because plasma chylomicron and triglycerides from the lipid infusion can disturb the colorimetric FFA assay, plasma samples taken during exogenous lipid infusion were filtered through a 0.22-μm Millipore filter (Millinex, Millipore BV, Tullagreen Carrigtwohill, County Cork, Ireland) before analysis.

2.8. Mitochondrial respiration

Permeabilized muscle fibers were obtained from the muscle biopsy following the protocol as described before [22,23]. Muscle bundles were blotted, and 5 mg (wet weight) was immediately transferred into the respiration chambers of the respirometer containing air-saturated Mir05 (respiration medium containing EGTA 0.5 μmol/L, potassium lactobionate 60 mmol/L, taurine 20 mmol/L, $MgCl_2.6H_2O$ 3 mmol/L, KH_2PO_4 10 mmol/L, Hepes 20 mmol/L, sucrose 110 mmol/L, and bovine serum albumin 1g/L).

Thereafter, the respiration chambers were hyperoxygenated and closed at an O_2 concentration of approximately 500 μmol/L. Oxygen limitation of respiration was prevented by maintaining the oxygen levels in the respirometer above air saturation in the range of 500 to 200 μmol/L(26).

Mitochondrial respiratory rates were measured at 37°C by polarographic oxygen sensors in a 2-chamber Oxygraph (OROBOROS Instruments, Innsbruck, Austria) equipped with a Peltier thermostat and electromagnetic stirrers. The oxygen concentration was recorded at 2.0-second intervals using the acquisition software DatLab 4 (OROBOROS Instruments). The first derivative of the oxygen tension changes is displayed as oxygen flux, and mean values during about 1 minute were obtained from these recordings for calculation of stable oxygen flux rates.

Ex vivo high-resolution respirometry made it possible to measure mitochondrial capacity under similar substrate conditions on both study days. A multisubstrate protocol was used reconstituting the operation of the tricarboxylic acid cycle and preventing depletion of key metabolites from the mitochondrial matrix [24]. First, malate (2 mmol/L) was added for depletion of endogenous substrates. Palmitoylcarnitine (50 μmol/L) was then added as a substrate for fatty acid oxidation. Adenosine diphosphate (ADP) was added to induce state 3 respiration (=ADP-stimulated respiration) fueled by malate and palmitoylcarnitine. Glutamate (20 mmol/L) and succinate (20 mmol/L) were subsequently added for additional electron input in, respectively, complex I and complex II. With these substrates, maximal physiological capacity is obtained, reconstituting the operation of the tricarboxylic acid cycle and preventing depletion of key metabolites from the mitochondrial matrix [24]. Cytochrome c (10 μmol/L) was added to check the integrity of the outer mitochondrial membrane. Maximal oxygen flux rates were measured with the chemical uncoupler carbonyl-cyanide-4-(trifluoromethoxy)-phenylhydrazone (FCCP), which was titrated (0.25 μmol/L per addition) until no further stimulation of respiration could be detected. Mitochondrial respiration measured ex vivo in permeabilized muscle fibers obtained from the same muscle biopsy can show some variation [24]. Therefore, all measurements were done in duplicate.

2.9. Mitochondrial content

Citrate synthase activity was measured in muscle biopsies homogenized in 100 mmol/L Tris.Cl (pH 8.0). Activity levels, expressed as micromoles per milligram total protein per minute, were measured spectrophotometrically according to the method of Srere [25].

2.10. Statistical analysis and calculations

Peripheral glucose uptake (rate of disappearance [Rd]) was calculated with modified forms of the Steele equations as described [26].

Data are presented as medians (range). Participants were their own controls; therefore, statistical evaluation of these repeated measures (paired observations) was calculated using the nonparametric Wilcoxon signed rank test. The SPSS statistical software analysis program version 12.0.2 (SPSS, Chicago, IL) was used for statistical analysis. $P < .05$ was regarded as statistically significant.

3. Results

Anthropometric data of the participants in this study are summarized in Table 1. Weight and body fat percentage of the participants did not differ between the 2 study days ($P = .64$ and $P = .61$, respectively).

3.1. Peripheral insulin sensitivity

The increase in plasma FFA concentration by an intravenous lipid emulsion during a 6-hour hyperinsulinemic euglycemic clamp strongly reduced peripheral glucose uptake by approximately 40%. Rate of disappearance was 40 (33-62) μmol/kg·min during intravenous lipid infusion vs 68 (50-87) μmol/kg·min during intravenous saline infusion ($P = .008$) (Fig. 1).

3.2. FFA, glucose, and insulin concentrations

Fasting plasma FFAs were not statistically different between both study days (Table 2). After 6 hours of hyperinsulinemia, plasma FFA concentrations were 0.48 (0.32-0.56) mmol/L during the lipid infusion, whereas they were all less than the detection limit of 0.02 mmol/L during the saline infusion. To avoid different levels of FFA during infusion of the lipid emulsion, we clamped plasma FFA at a physiological level of 0.5 mmol/L. Therefore, the lipid infusion rate differed. The total volume of the lipid emulsion infused during 6 hours was 698 (317-1302) mL.

Table 1 – Anthropometric data

Age (y)	23 (19-30)
Body mass index (kg/m^2)	21.4 (19.6-25.7)
Body fat (%)	14.0 (11.3-25.5)
Fasting plasma glucose (mmol/L)	4.7 (3.8-5.2)
Fasting plasma insulin (pmol/L)	32 (17-48)
Vo_{2max} (mL/min·kg)	54 (49-57)

Data are expressed as median (range). N = 9.

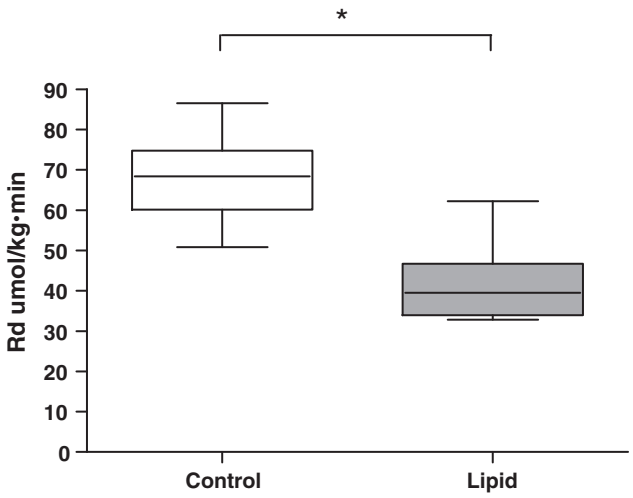

Fig. 1 – Peripheral glucose uptake (Rd) after 6 hours of supraphysiological insulin concentrations with a concomitant 6-hour lipid infusion (gray bar) compared with saline (white bar) (N = 9). *P = .008, Wilcoxon signed rank test. Box plots represent median, 25th percentile, and 75th percentile and minimum and maximum.

Glucose and insulin concentrations during the clamp did not differ during the lipid infusion compared with the saline infusion (Table 2).

3.3. Glucose and fatty acid oxidation

Resting energy expenditure at the end of the clamp was significantly higher after intravenous lipid compared with saline infusion. This is explained by providing higher caloric supply during the lipid compared with the control saline infusion. Glucose oxidation rate was significantly lower, whereas fat oxidation rate was significantly higher, during the lipid infusion (Table 2).

3.4. Mitochondrial respiration

Various aspects of mitochondrial respiration were determined in permeabilized muscle fibers of all participants. The results are depicted in Fig. 2.

Table 2 – Measurements during 6-hour hyperinsulinemic euglycemic clamp

	Saline	Intralipid	P
Glucose (mmol/L)	5.1 (4.8-5.3)	5.1 (4.9-5.3)	.33
Insulin (pmol/L)	560 (470-740)	593 (485-789)	.17
Fasting FFA (mmol/L)	0.33 (0.21-0.73)	0.47 (0.13-0.60)	.59
FFA clamp (mmol/L)	<0.02	0.48 (0.32-0.56)	<.001
REE (kcal/d)	1928 (1832-2356)	2068 (1899-2371)	.04
Glucose oxidation (mg/kg·min)	3.32 (2.70-4.04)	1.59 (0.25-2.90)	.017
Fat oxidation (mg/kg·min)	0.58 (0.36-0.78)	1.25 (0.85-1.66)	.012

Data are expressed as median (range). N = 9. Statistical analysis was performed using the Wilcoxon signed rank test.

The addition of malate and palmitoylcarnitine did not stimulate oxygen consumption before the addition of ADP (lipid 4.02 [3.18-6.07] and saline 3.90 [2.95-6.36] pmol/mg·s) (P = .77). The addition of ADP and thereafter sequential addition of the other substrates to the muscle tissue resulted in a stepwise increment in respiration. The ADP-stimulated respiration rates fueled by malate and palmitoylcarnitine were not different between both conditions (lipid 21.34 [18.0-23.7] vs control 21.3 [14.9-24.6] pmol/mg·s) (P = .39). Subsequent addition of glutamate increased respiration similarly in both conditions (lipid 35.4 [30.1-42.8] vs control 38.0 [29.7-48.9] pmol/mg·s) (P = .26)). The addition of succinate increased the respiration rate further; but again, the rise was not different between both conditions, resulting in similar state 3 rates (lipid 54.5 [46.3-69.3] vs control 55.4 [47.0-71.4] pmol/mg·s, respectively) (P = .31). The addition of cytochrome c did not increase oxygen consumption, indicating that the mitochondrial outer membrane was intact. Finally, maximally uncoupled respiration, indicating the maximal capacity of the electron transport chain, was achieved upon the addition of the chemical uncoupler FCCP; but again, these rates of respiration were not different between both conditions (lipid 104.67 [77.4-111.1] vs control 96.1 [79.6-115.4] pmol/mg·s) (P = .78).

When mitochondrial respiration rates were corrected for citrate synthase activity, similar results were obtained (data not shown).

3.5. *Mitochondrial content in muscle biopsies*

Correction for mitochondrial density is important because it has been shown that this may explain some of the differences found between controls and patients with DM2 [22]. In addition, mitochondria from different muscle fiber types have different metabolic characteristics and oxidative capacities [27,28]. To circumvent these confounding factors, each participant served as his own control. Furthermore, participants were instructed to maintain the same level of physical activity during the total study period, as the level of physical activity is one of the main determinants for mitochondrial capacity [29]. In addition, we measured $V_{O_{2max}}$ to exclude subjects with low levels of physical fitness.

Citrate synthase activity can be used as a marker of cellular content of mitochondria [22,27]. We therefore determined activity of this enzyme in the biopsies from each participant during both infusions. No difference in citrate synthase activity was found (lipid 2.33 [1.53-5.16] vs control 2.15 [1.02-4.34] μmol/mg protein per minute) (P = .44). When respiratory fluxes were corrected for citrate synthase activity, the comparison between the 2 infusion conditions as discussed above did not change (data not shown).

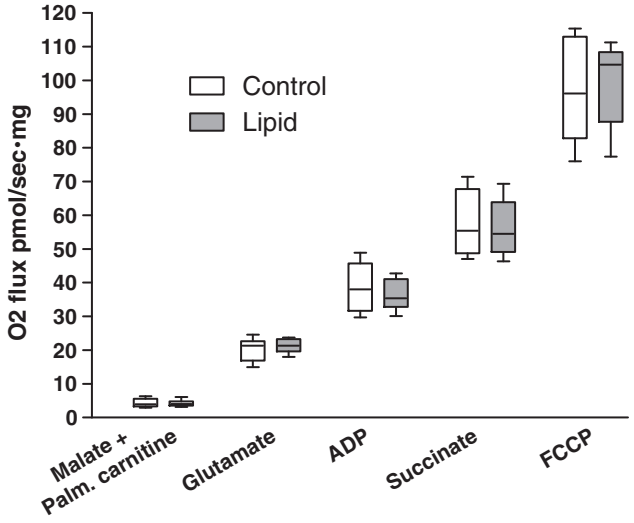

Fig. 2 – Mitochondrial respiration rates. Respiration fluxes per milligram wet muscle weight during Intralipid (gray bars) infusion or control saline (white bars), after the subsequent addition of malate and palmitoylcarnitine, ADP, glutamate, succinate, and FCCP (N = 9). Box plots represent median, 25th percentile, and 75th percentile and minimum and maximum. No statistically significant differences were observed between groups.

4. Discussion

This study demonstrates for the first time that, in healthy lean men, short-term elevation of plasma FFA within the physiological range during hyperinsulinemia does not affect intrinsic skeletal muscle mitochondrial respiratory capacity, whereas peripheral glucose uptake decreases by approximately 40%. Indeed, when the FFA-induced insulin resistance was accompanied by FA-induced mitochondrial damage, parameters of mitochondrial respiration in muscle fibers should have been altered.

The association between mitochondrial function and insulin resistance is controversial [30-32]. Recently, the complex relationship between excess lipid exposure, mitochondrial dysfunction, and insulin resistance in human skeletal muscle has been reviewed [33]. At present, it cannot be definitively concluded that, in obese insulin-resistant subjects, mitochondria have an intrinsic defect (dysfunction in the respiratory chain) [34]. Instead, decreased mitochondrial capacity in obese insulin-resistant subjects could be a reflection of decreased physical activity [35], mitochondrial content [22], and muscular blood flow [36]. A crucial issue remains whether increased lipid availability can indeed primarily affect mitochondrial function.

To investigate the effect of increased FFA on intrinsic mitochondrial function, we carried out detailed studies on mitochondrial respiration in permeabilized muscle fibers. By titration of various substrates, we stimulated the tricarboxylic acid (TCA) cycle and the different complexes of the electron transport chain. Basal- and ADP-stimulated oxygen consumption rates as well as maximal uncoupled oxidative capacity induced by FCCP (making oxygen consumption independent of ATP production) were almost identical on both study days. However, because we did not measure ATP production directly, possible differences in mitochondrial uncoupling induced by fatty acids [37] may have masked a potentially lower capacity for ATP production in the high-lipid condition. Nonetheless, in the case of (substantial) uncoupling, respiration in the absence of ADP would have been higher in the lipid-infused subjects; and this was not observed. In agreement with this, a recent study showed that the mitochondrial uncoupling proteins UCP2 and

UCP3 were not upregulated after a 6-hour increase of plasma FFA induced by a lipid infusion [38]. Furthermore, we performed a lipid infusion during a concomitant hyperinsulinemic euglycemic clamp; and it has been shown that hyperinsulinemia abolishes effects on UCP3 expression induced by FFA [39].

Differences in mitochondrial ATP production between obese subjects with DM2 and control subjects may be caused by impaired insulin-stimulated production of ATP as a result of reduced insulin signaling, as there are no differences under low insulinemic conditions [40,41]. In the present study, muscle biopsies were taken after 6 hours of increased FFA during hyperinsulinemic euglycemic conditions. High insulin levels stimulate mitochondrial ATP synthesis and production of key mitochondrial proteins. During lipid infusion, insulin signalling is impaired, most likely via accumulation of muscle cytosolic concentrations of different FA metabolites [42,43]. Brehm et al [16] showed that experimental elevation of FFA by a lipid infusion during a hyperinsulinemic clamp in young healthy subjects impaired insulin-stimulated in vivo ATP synthesis in skeletal muscle. They hypothesized that this impairment could have been caused by a difference in insulin signaling induced by high FFA, in energy demand, or in substrate availability. In fact, this hypothesis seems to be confirmed by the findings of the present study because we show that, during similar insulin-resistant conditions, mitochondrial respiration capacity ex vivo and under standardized substrate and ADP conditions was not affected compared with the control situation. This makes mitochondrial damage [12,13] a less likely cause of the described decreased ATP synthesis.

This observation is also strengthened by the finding that only longer-term exposure to high levels of plasma FFA during hyperinsulinemia induces a negative effect on ATP production measured in vivo, whereas shorter-term exposure, that is, less than 3 hours, shows no effect [17]. It is known that FFA-induced peripheral insulin resistance is dose and time dependent, with the most profound effect occurring after 6 hours [2]. This suggests, together with our findings, that reduced insulin-stimulated ATP synthesis during high levels of FFA is secondary to insulin resistance and that this reduction in function is not explained by intrinsic mitochondrial defects.

Lipid infusion without concomitant hyperinsulinemia in healthy young men has been shown to reduce mitochondrial membrane potential without altering gene expression, mitochondrial morphology, or content [38]. Functional measurements of ATP synthesis or oxygen consumption were not performed in this study. Moreover, a tendency toward a greater decline in ATP content during the control saline infusion was described. This questions whether intrinsic mitochondrial function was affected. Because other parameters were not altered, it was hypothesized that FFAs may functionally impair mitochondrial function, which should be reversible after the lipid challenge is withdrawn. Mitochondrial activity depends on energy demand and substrate availability. The advantage of ex vivo mitochondrial respirometry in the present study was that mitochondrial capacity after exposure to increased FFAs was measured under similar substrate and ADP conditions. Because of the permeabilization and washing steps, the in vivo effects of FFAs may be abolished. The lower membrane potential reported [38] could reflect a functional impairment, which is completely reversible when the lipid challenge is withdrawn. However, how FFAs lower the mitochondrial membrane potential and whether this really reflects a functional impairment need to be further established. That mitochondrial gene expression, morphology, and content were not altered in this study [38] is in agreement with our finding that intrinsic mitochondrial capacity is not affected upon a 6-hour lipid infusion.

A 6-hour elevation of plasma FFA may be too short to induce detrimental changes on mitochondrial biogenesis and intrinsic capacity, as it has been shown that expression of genes that are involved in mitochondrial functioning does not alter over this short period [11,33], whereas prolonged exposure of 48 hours or 3-day high-fat feeding in healthy men does decrease expression of these genes [10,44].

These short-term studies do not necessarily reflect or are representative for the chronic elevation of FFA in obese insulin-resistant subjects. To really answer the question of whether mitochondrial dysfunction plays a role in the development of obesity-induced insulin resistance, it would be interesting to evaluate mitochondrial morphology, gene expression, and intrinsic capacity during the development of obesity. These studies have been done in rodents. Hancock et al [31] showed that 4- to 5-week high-fat feeding in rats results in an increase in mitochondria and in the capacity of muscle to oxidize fat, concomitantly with the development of muscle insulin resistance. In a prolonged study, a 16-week high-fat diet in rats did not induce differences in mitochondrial function or excess superoxide production in all insulin-sensitive tissues [45], whereas a high-fat, high-caloric diet in mice did alter mitochondrial biogenesis, structure, and function in muscle tissue, but only after 16 weeks [46]. The onset of the mitochondrial alterations did not precede the onset of the insulin resistance, which was already present in a much earlier state. Furthermore, the increase in muscle reactive oxygen species (ROS) production, which could induce mitochondrial damage, occurred specifically after 16 weeks of the hypercaloric diet, when the mice were already hyperglycemic and hyperlipidemic [46].

In conclusion, short-term elevation of plasma fatty acids within the physiological range in the presence of hyperinsulinemia induces peripheral insulin resistance, but does not disturb intrinsic mitochondrial capacity in skeletal muscle.

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