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# Exercise-induced, but not creatine-induced, decrease in intramyocellular lipid content improves insulin sensitivity in rats☆

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**Abstract**

The effect of creatine supplementation, alone or in combination with exercise training, on insulin sensitivity, intramyocellular lipid content (IMCL) and fatty acid translocase (FAT)/CD36 content was investigated in rats fed a sucrose-rich cafeteria diet during 12 weeks. Five experimental conditions were CON, receiving normal pellets; CAF, fed the cafeteria diet; $CAF_{TR}$, fed the cafeteria diet together with exercise training in weeks 8–12 and $CAF_{CR}$ and $CAF_{CRT}$ that were analogous to CAF and $CAF_{TR}$, respectively, but which received daily 2.5% of creatine monohydrate. During intravenous glucose tolerance test, compared with CON, whole-body glucose tolerance was reduced in CAF and $CAF_{CR}$ but not in $CAF_{TR}$ and $CAF_{CRT}$. Insulin-stimulated glucose transport in perfused red gastrocnemius muscles was impaired in CAF and $CAF_{CR}$ but not in the trained groups. IMCL content in soleus and extensor digitorum longus muscles was higher in CAF than in CON, but not in $CAF_{TR}$, $CAF_{CR}$ and $CAF_{CRT}$. Compared with CON and CAF, FAT/CD36 protein content in m. soleus, was ~40% lower in $CAF_{CR}$, $CAF_{TR}$ and $CAF_{CRT}$. The fraction of fecal fat, as determined in a 3-week post hoc study, was 25% higher in $CAF_{CR}$ than in CON. Moreover, in $CAF_{CR}$, triglyceride concentration in blood and liver were significantly lower than in CAF. It is concluded that creatine supplementation in rats on a cafeteria diet inhibits IMCL accumulation via inhibition of gastrointestinal lipid absorption together with lower muscle FAT/CD36 content. Furthermore, exercise-induced but not creatine-induced reduction of IMCL is associated with improved insulin action on glucose transport in muscle cells.


*Keywords:* Glucose metabolism; Exercise; Fatty acid translocase/CD36; Muscle

## 1. Introduction

Since Harris et al. in 1992 reported that oral creatine supplementation can elevate creatine content in human muscles [1], creatine has become one of the most popular nutritional supplements to boost performance in athletes. In the meantime, the "ergogenic" effects of creatine supplementation in healthy individuals are well documented [2-6], and this triggered interest in the potential of creatine as a therapeutic agent to improve muscular function in the elderly or in patients afflicted by neuromuscular disease. In this regard, creatine supplementation was found to increase creatine content in these potential target populations [7,8], yet available data with regard to the effects on muscular functional capacity are equivocal [7-8], and evidence for long-term therapeutic efficacy is lacking.

Some literature data [9] indicate that creatine intake can beneficially impact glucose metabolism. Theoretically, creatine supplementation could improve whole body glucose homeostasis either by stimulating pancreatic insulin secretion [10] or by facilitating peripheral glucose disposal. With regard to the latter mechanism, supplementary creatine intake can increase muscle glycogen concentration in both humans [11-13] and rats [14]. In some studies, creatine intake also improved whole body glucose tolerance. Also, in a rat model of inherited Type 2 diabetes, creatine administration lowered the plasma insulin response during an intravenous glucose tolerance test despite equal glucose clearance [15]. Furthermore, we previously demonstrated that creatine intake in healthy volunteers counteracted the fall of muscle glucose GLUT4 protein expression during leg immobilization [16]. Along this line, we and others also found that exercise training is an important co-factor for creatine supplementation to translate into either increased muscle GLUT4 protein content or glycogen level, or improved glucose tolerance [11,12,17]. Still, to date the precise physiological and molecular mechanisms by which creatine intake can impact on glucose homeostasis are largely unclear.

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The present study was set up to further investigate the effects of creatine supplementation, alone or in combination with exercise training, on whole body glucose homeostasis and muscular insulin sensitivity in rats. We administered a cafeteria diet known to induce obesity and a state of glucose intolerance together with muscular insulin resistance in rats [18,19]. We anticipated that cafeteria diet would increase intramyocellular lipid content (IMCL) by increased delivery of fatty acids to muscle cells [20]. Studies in both rats and humans have clearly shown that conditions where fatty acid delivery to muscle cells exceeds the potential for intracellular fat oxidation, cause IMCL accumulation together with deficient insulin action [21-24]. Therefore, we were also specifically interested to investigate the relationship between the changes in muscular insulin sensitivity and IMCL content induced by the administration of cafeteria diet on the one hand, and creatine supplementation and exercise training on the other hand. We hypothesized that creatine supplementation in conjunction with exercise training can negate the detrimental effects of cafeteria diet on IMCL content, muscular insulin resistance and whole body glucose homeostasis. Partial results of this study have been previously reported elsewhere [25,26].

## 2. Materials and methods

### 2.1. Animals

Male Wistar rats, weighing 200–225 g, were obtained from K.U.Leuven animal breeding centre. Rats were housed in individual cages and maintained on a constant light-dark cycle (12 h: 12 h) at 22°C. The rats were given ad libitum access to food and water. This study protocol was approved by the animal Ethics Committee of K.U. Leuven.

### 2.2. Study protocol

Part of the study protocol has been previously reported in detail elsewhere [25]. Briefly, rats were randomly assigned to five experimental groups receiving different dietary and exercise interventions during a 12-week period. Group 1 (CON) served as a control group and received a normal rodent pellet diet (Muracon-G, Carfil Quality, Oud-Turnhout, Belgium) throughout the study. Group 2 (CAF) was fed a so-called cafeteria diet [19], the detailed composition of the two diets is given in Table 1. Group 3 ($CAF_{CR}$) was similar to CAF, yet the cafeteria diet was supplemented with 2.5% creatine monohydrate (Sigma-Aldrich, St. Louis, MO, USA). Group 4 ($CAF_{TR}$) also received cafeteria diet similar to CAF but, in addition, was subjected to exercise training (1 h swimming, five times per week in water maintained at 32–35°C) during the last 4 weeks of the dietary intervention period. To ensure continuous swimming a load equivalent to 2% of body weight was attached to the tail. Group 5 ($CAF_{CRT}$) received the cafeteria diet supplemented with 2.5% creatine monohydrate and was also subjected to the same exercise training as $CAF_{TR}$. The rats were provided with ample freshly prepared cafeteria food (60 g; CAF, $CAF_{CR}$, $CAF_{TR}$, $CAF_{CRT}$) or normal pellets (40 g; CON) every morning and daily food intake was calculated by weighing the residual food the next morning. At the end of the 12-week intervention period and after an overnight fast, an intravenous glucose tolerance test (IVGTT) was performed. The rats were then allowed to recover from the IVGTT and received their specific experimental diet for 4 more days. In addition, $CAFT_{TR}$ and $CAF_{CRT}$ rats performed two more training sessions. Following an overnight fast and ~48 h following the last training session, hind limb perfusions were performed to assess muscle glucose transport.

Table 1
Energy composition of the control and cafeteria diet

| | Control diet | Cafeteria diet |
|---|---|---|
| Total carbohydrate | | |
| Starch | 55% | 21% |
| Sugars | 8% | 48% |
| Total fat | | |
| Saturated FFA | 3% | 8% |
| Unsaturated FFA | 9% | 8% |
| Total protein | 25% | 15% |

The energy content (% total kcal) of fat, carbohydrates, and protein of the control and cafeteria diets is given.

### 2.3. Intravenous glucose tolerance tests

Rats were anaesthetized by an intraperitoneal injection (0.2 ml/100 g body weight) of a mixture containing 50% Ketalar (50 $mg.ml^{-1}$ ketamine, Pfizer, Brussels, Belgium), 25% Rompun (2% xylazine, Bayer, Leverkussen, Germany) and 25% atropine (0.5 $mg.ml^{-1}$), and prepared surgically for an IVGTT. This involved catheter insertion into the left jugular vein. Subsequently, after an overnight fast (16-18 h) conscious rats were injected with 1 g $glucose.kg^{-1}$ body weight using a 30% ($w.v^{-1}$) glucose solution in 0.9% ($w.v^{-1}$) saline. Tail vein blood samples (25 μl) were collected at regular intervals for glucose and insulin determination, and the insulinogenic index was calculated as the paired ratio between plasma insulin and blood D-glucose concentration, as previously described [15,25]. Total area under the curve (AUC) for glucose and insulin was calculated using trapezoidal rule.

### 2.4. Rat hind limb perfusions

To evaluate the effects of cafeteria diet, creatine supplementation and training on muscle glucose transport, at the end of the 12-week intervention period and after an overnight fast, rats were anaesthetized by an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body weight) and were prepared surgically for hindquarter perfusion as previously described in detail [27]. The perfusate (500 ml at 35°C) consisted of a Krebs-Ringer bicarbonate buffer solution, 4% bovine serum albumin (fraction V, Sigma-Aldrich), 0.15 mM pyruvate, 4.2 $IU.ml^{-1}$ heparin, and 200 $\mu U.ml^{-1}$ insulin. Furthermore, 2-deoxy-D-glucose (8 mM), mannitol (1 mM), as well as 2-deoxy-D-2,6[$^3$H]glucose (Amersham Radiochemicals, GE Healthcare, Uppsala, Sweden; specific activity 51.0 $mCi^{-1}.mmol.L^{-1}$) and $D^{-1}$-[$^{14}$C]mannitol (Amersham Radiochemicals; specific activity 87.0 $mCi.mmol^{-1}.L^{-1}$) were added in order to obtain constant perfusate activities of 0.075 $\mu Ci.ml^{-1}$ for $^3$H and 0.050 $\mu C.ml^{-1}$ for $^{14}$C during a 10 min one-way perfusion at a flow of 20 $ml.min^{-1}$ . At the end of the perfusion, a medial superficial part of m. gastrocnemius (white gastrocnemius), a deep proximal and medial portion of m. gastrocnemius (red gastrocnemius), m. extensor digitorum longus (EDL) and m. soleus were dissected from both hind limbs and freed from visible connective tissue and blood. Muscle samples from one limb were freeze clamped with aluminum clamps cooled in liquid nitrogen. Part of this sample was used for determination of muscle glucose transport, using perchloric acid extracts and applying correction for $^3$H for label in the extracellular space as determined by the $^{14}$C counts for mannitol, as previously described in detail [28]. Muscle samples from the other limb were mounted in embedding medium (Tissue-Tek, Sakura FineTek, Zoeterwoude, The Netherlands) before being frozen in isopentane cooled in liquid nitrogen. All samples were stored at −80°C until analysis at a later date.

### 2.5. Histochemical analyses on muscle samples

Serial sections (4 μm) from soleus and EDL muscles were laid together on uncoated glass slides and fiber-specific staining for the intramyocellular lipid content (IMCL) was carried out using the Oil-Red-O (ORO) technique as previously described in detail [29]. Fiber type-specific IMCL content was expressed as the percentage of fiber area covered by stained fat droplets, and the mean values were used for data analysis.

### 2.6. Biochemical analyses on muscle samples

For determination of muscle FAT/CD36 protein content, frozen samples (30–40 mg) from soleus and EDL muscles were homogenized in 400 μl of lysis buffer (50 mM Hepes pH 7.5; 150 mM NaCl; 20 mM $Na_4P_2O_7$; 20 mM β-glycerophosphate; 10 mM NaF; 2 mM EDTA; 1 mM $MgCl_2$; 1 mM $CaCl_2$; 2 mM $Na_3VO_4$; 1% ($v.v^{-1}$) Igepal; 10% ($v.v^{-1}$) glycerol and one tablet/10 ml Protease Inhibitor Cocktail Tablets, EDTA-free (Roche Applied Science, Basel, Switzerland)). After incubation at 4°C for 30 min, samples were centrifuged (4°C, 60 min; 13,000×g), and the supernatant was stored at −80°C. Total protein concentration was determined using the bicinchoninic acid Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Relative protein level of FAT/CD36 was evaluated by Western blotting. Briefly, 35 μg protein from each sample was separated by SDS-PAGE using precast Criterion XT 10% Bis-Tris gels (Bio-Rad, Hercules, CA, USA). After the proteins were transferred to a polyvinylidene fluoride membrane (Bio-Rad), the membrane was blocked under gentle agitation at room temperature for 1 h in 3% bovine serum albumin (ICN Biomedical, Aurora, OH, USA) in Tris-buffered saline (TBS)+0.1% Tween 20 (ICN Biomedical). Blocked membranes were incubated overnight at 4°C 5% bovine serum albumin in TBS+0.1% Tween 20 and a primary rabbit polyclonal to FAT/CD36 antibody (Abcam, Cambridge, MA, USA). Next morning, a Polyclonal goat anti-rabbit immunoglobulin alkaline phosphatase-conjugated antibody (Dako, Glostrup, Denmark) diluted in TBS-Tween 20 was added for 1 h. Finally, bands were visualized with a chemifluorescence substrate (Roche Diagnostics, Mannheim, Germany) and quantified by means of a Kodak Image Station 440 CF (Leipzig, Germany) running with Kodak ID image analysis software. To correct the inter-gel differences, band intensities were expressed relative to a control pool which was composed of all control samples and was run together with the target samples. Muscle free creatine, phosphocreatine and glycogen contents were assayed by standard enzymatic fluorometric assays as previously described [30]. Muscle total creatine content was calculated as the sum of free creatine and phosphocreatine contents.

Table 2
Body weight gain and food intake in control rats and in rats receiving cafeteria diet

| | CON | CAF | $CAF_{CR}$ | $CAF_{TR}$ | $CAF_{CRT}$ |
|---|---|---|---|---|---|
| Weight gain (g) | 255±14 | 305±10* | 315±19* | 253±7† | 264±15† |
| Daily food intake (g) | 31.83±0.45 | 41.98±0.73* | 42.31±0.80* | 38.89±0.49*† | 43.84±0.64* |
| Fats (g/day) | | | | | |
| Unsaturated | 0.82±0.01 | 0.80±0.01 | 0.80±0.01 | 0.74±0.01*† | 0.84±0.01 |
| Saturated | 0.28±0.01 | 0.88±0.02* | 0.88±0.02* | 0.82±0.01*† | 0.92±0.01* |
| Proteins (g/day) | 5.25±0.08 | 3.41±0.06* | 3.41±0.06* | 3.16±0.04*† | 3.55±0.04* |
| Carbohydrates (g/day) | | | | | |
| Sugar | 1.72±0.02 | 11.2±0.20* | 11.3±0.20* | 10.4±0.13*† | 11.7±0.16* |
| Starch | 11.3±0.16 | 4.92±0.09* | 4.96±0.09* | 4.56±0.06*† | 5.10±0.07* |

Values are means±S.E. for control rats (CON; $n$=10), rats receiving cafeteria diet alone (CAF; $n$=16), or supplemented with 2.5% creatine ($CAF_{CR}$; $n$=19), and rats receiving cafeteria diet in conjunction with exercise training alone ($CAF_{TR}$; $n$=15) or in combination with creatine supplementation ($CAF_{CRT}$; $n$=9).
*$P$<.05 versus CON; †$P$<.05 versus CAF.

### 2.7. Blood and liver triglyceride levels and fecal fat content

In a later stage of the study, post hoc experiments were set up in a subgroup of rats to evaluate the short-term effects of cafeteria diet, alone or in combination with creatine, on plasma and hepatic triglyceride concentrations and fecal fat content. Rats were randomly assigned to CON ($n$=5), CAF ($n$=5) or $CAF_{CR}$ ($n$=5) for a period of 3 weeks. Rats were permanently housed in individual cages adapted for collection of feces. Feces were collected during a 24-h period on Days 7, 14 and 21 of the dietary treatment. Samples were freeze-dried for 48 h at −50°C, pulverized and stored at −20°C until analysis on a later date. Fecal fat in lyophilized fecal samples was measured gravimetrically after extraction in petroleumether using an automatic Soxhlet analyzer (Soxtec 2050, Foss, Amersfoort, the Netherlands). Therefore, about 400 mg of lyophilized feces was carefully weighed into a cellulose thimble which was mounted in the analyzer. The extraction cycle consisted of 4 consecutive steps including boiling (1 h), rinsing (2 h), evaporation of the solvent (30 min) and drying (40 min). After drying, the dry fat was weighed and the fecal fat content was calculated as percentage of lyophilized fecal mass. All measurements were performed in duplicate.

At the end of the 3-week dietary intervention and after an overnight fast, blood was collected from a tail vein. The blood was directly put on a test strip (Accutrend Triglycerides, Roche Diagnostics) for determination of blood triglyceride concentration using a micro-autoanalyzer (Accutrend Plus, Roche Diagnostics). After the 3-week intervention period, the rats were anaesthetized by an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body weight), and the livers were removed, freeze-clamped with aluminum clamps cooled in liquid nitrogen and stored at −80°C until analysis. About 50 mg of liver was homogenized in 3.8 ml $CH_3OH/CHCl_3/H_2O$ (2:1:0.8) (v/v/v) using a Polytron homogenizer (Kinematica, Lucerne, Switzerland). Total lipids were isolated by Bligh and Dyer extraction [31], using 1 M NaCl for phase separation and washing. After wet ashing of 100 µl of the total lipid extract, the amount of inorganic phosphate was determined [32]. A $KH_2PO_4$ standard curve was set up (0–100 nmol) to allow calculation of the phospholipid content in the samples. Triglycerides were isolated by thin layer chromatography and analyzed with coupled enzymatic assays as described before [33] except that triglycerides were hydrolyzed chemically in the presence of 0.5 M KOH in 90% (v/v) ethanol for 60 min at 75°C.

### 2.8. Statistical analysis

Statistical evaluation of the data was performed using one-way analysis of variance (ANOVA) or two-way ANOVA with repeated measures (Statistica, Statsoft) followed by a planned contrast analysis was used for post hoc comparisons when appropriate. Data were presented as mean±S.E. A probability level ($P$) of ≤.05 was chosen as the threshold for acceptance of statistical significance.

## 3. Results

### 3.1. Characteristics of the dietary interventions and effect on body weight (Table 2)

Compared with CON, energy intakes over the 12-week intervention period were ~12–15% higher in CAF, $CAF_{CR}$ and $CAF_{CRT}$ ($P$<.05). In $CAF_{TR}$, energy intake was ~4% higher than in CON ($P$=.08) but still slightly lower than in CAF (–7%, $P$ < .05). Due to different dietary compositions, intakes ($g.day^{-1}$) of carbohydrates, fats and proteins also were different between the experimental groups (Table 2). Compared with CON, all groups receiving cafeteria diet ingested about threefold more saturated fats ($P$<.05) and fivefold greater amount of sugars ($P$<.05). However, they ingested 30–40% less protein and 55–60% less starch ($P$ < .05). The higher energy intake in CAF, at the end of the intervention period, resulted in ~20% higher body weight gain (Table 2; $P$<.05) and ~12% higher body weight (522±10g; $P$<.05) than in CON (474±16 g). Compared with CON, in $CAF_{CR}$, body weight was increased to the same degree (530±18 g, $P$<.05) as in CAF, but in the training groups, body weight gains (Table 2) and final body weights ($CAF_{TR}$, 471±7 g; $CAF_{CRT}$, 479±15 g) were similar to CON. Moreover, epididymal fat mass was increased in CAF (15.2±1.0 g,

Table 3
Effect of cafeteria diet, creatine and training on blood glucose and plasma insulin concentrations during an IVGTT

| | | CON | CAF | $CAF_{CR}$ | $CAF_{TR}$ | $CAF_{CRT}$ |
|---|---|---|---|---|---|---|
| 0 min | Glucose | 3.30±0.15 | 3.66±0.16 | 3.32±0.09 | 3.59±0.17 | 3.20±0.08 |
| | Insulin | 0.90±0.14 | 2.52±0.25[a] | 2.18±0.18[a] | 1.50±0.12[b] | 1.25±0.18[b] |
| | IG | 0.27±0.04 | 0.70±0.06[a] | 0.64±0.05[a] | 0.42±0.04[b] | 0.30±0.06[b] |
| 10 min | Glucose | 9.42±0.63 | 11.11±0.57 | 10.66±0.35 | 10.86±0.35 | 9.69±0.25 |
| | Insulin | 4.79±0.63 | 10.20±0.83[a] | 10.34±0.76[a] | 7.40±1.10[b] | 7.05±1.19[b] |
| | IG | 0.53±0.07 | 0.99±0.10[a] | 1.07±0.08[a] | 0.73±0.11[b] | 0.52±0.06 |
| 30 min | Glucose | 5.63±0.56 | 7.35±0.61[a] | 5.86±0.37 | 6.59±0.50 | 5.23±0.41 |
| | Insulin | 1.47±0.30 | 4.36±0.48[a] | 3.51±0.36[a] | 2.26±0.16[b] | 1.84±0.32[b] |
| | IG | 0.28±0.03 | 0.59±0.04[a] | 0.61±0.05[a] | 0.38±0.05[b] | 0.27±0.05[b] |
| 90 min | Glucose | 3.57±0.20 | 4.39±0.28 | 4.12±0.15 | 4.19±0.23 | 3.92±0.32 |
| | Insulin | 1.27±0.33 | 3.36±0.41[a] | 2.76±0.24[a] | 1.78±0.16[b] | 1.69±0.30[b] |
| | IG | 0.36±0.08 | 0.75±0.07[a] | 0.64±0.06[a] | 0.43±0.04[b] | 0.32±0.06[b] |
| AUC | Glucose | 579±29 | 721±44[a] | 648±34 | 696±36[a] | 607±33[b] |
| | Insulin | 185±28 | 443±39[a] | 390±43[a] | 264±22[b] | 239±36[b] |

Values are mean±SE and are expressed in $mmol.l^{-1}$, $ng.ml^{-1}$, $mg.mol^{-1}$, $mmol.l^{-1}.min^{-1}$ and $ng.ml^{-1}.min^{-1}$ for glucose, insulin concentrations, insulinogenic index (IG), AUC for glucose and insulin, respectively. Glucose and insulin concentrations were evaluated in the minutes of 0, 10, 30 and 90 during IVGTT in 5 intervention groups: control group (CON; $n$=12), rats receiving cafeteria diet alone (CAF; $n$=21), or in the presence of training interventions during the final 4 weeks diet ($CAF_{TR}$; $n$=15) and two groups receiving cafeteria diet supplemented with creatine without ($CAF_{CR}$; $n$=27) or with training interventions in the last 4 weeks of the study ($CAF_{CRT}$; $n$=11).
[a]$P$<.05 compared with CON; [b]$P$<.05 compared with CAF.

$n$=10, $P$ < .05) and $CAF_{CR}$ (12.5±1.3g, $n$=13, $P$ < .05) but not in $CAF_{TR}$ (10.5±0.9g, $n$=11) and $CAF_{CRT}$ (9.9±0.8 g, $n$=6), when compared with CON ($n$=4). Interestingly, epididymal fat mass was significantly higher ($P$=.0506) in CAF than in $CAF_{CR}$.

### 3.2. Whole-body glucose tolerance

Whole-body glucose tolerance was evaluated by means of a 2 h IVGTT (Table 3). At the end of the 12-week intervention period, fasting blood glucose concentrations were similar between the groups. However, compared with CON (0.90±0.14 ng.ml$^{-1}$), fasting plasma insulin level was markedly higher ($P$<.05) in CAF. This increase was blunted by exercise training ($P$ < .05), but not by creatine supplementation. Intravenous glucose injection at the start of the IVGTT rapidly increased blood glucose concentration to ~10 mmol·l$^{-1}$ at 10 min in all groups, where after values gradually decreased. Blood glucose values were not significantly different between the groups at any time, except at 30 min there was a significant difference between CAF and CON. Glucose infusion obviously increased plasma insulin, which peaked at ~10 min post glucose infusion. However, plasma insulin increased markedly more upon glucose infusion in CAF and $CAF_{CR}$ than in CON ($P$<.05). Furthermore, compared with CAF, exercise training suppressed the glucose-induced increase in plasma insulin in both $CAF_{TR}$ and $CAF_{CRT}$ ($P$ < .05), yielding plasma insulin values which were not significantly different from CON. Along the differential blood glucose and plasma insulin responses between the groups, the insulinogenic index (Table 3) throughout the IVGTT and the ratios between the AUC for insulin and glucose ($AUC_{ins}/AUC_{glu}$) were significantly higher in CAF ($AUC_{ins}/AUC_{glu}$=0.62±0.04) and $CAF_{CR}$ ($AUC_{ins}/AUC_{glu}$=0.59±0.06) than in CON ($AUC_{ins}/AUC_{glu}$=0.32±0.04; $P$ < .05)), whilst in $CAF_{TR}$ ($AUC_{ins}/AUC_{glu}$=0.40±0.04) and $CAF_{CRT}$ ($AUC_{ins}/AUC_{glu}$=0.41±0.07), values were lower than in CAF ($P$ < .05).

### 3.3. Insulin-stimulated muscle glucose transport

Muscle glucose transport was assessed in rat muscles perfused at a submaximal insulin concentration of 200 μU.ml$^{-1}$ (Table 4). Compared with CON, glucose transport rate in CAF and $CAF_{CR}$ was reduced by about 60% in both white and red gastrocnemius muscles. Exercise training reversed the cafeteria diet-induced impairment of insulin-stimulated glucose transport in red gastrocnemius, but not in white gastrocnemius. Glucose transport in red gastrocnemius in both training groups ($CAF_{TR}$ and $CAF_{CRT}$), was about twofold higher ($P$ < .05) than in CAF, and values were not significantly different from CON. Neither cafeteria diet nor the training intervention significantly impacted on glucose transport rate in m. soleus. None of the interventions significantly impacted on muscle glucose transport in the absence of insulin (data not shown [26]).

Table 4
Effect of cafeteria diet, creatine and training on insulin-stimulated glucose transport in perfused hindlimb muscles

| | 200 μU. ml$^{-1}$ insulin | | |
|---|---|---|---|
| | WG | RG | SOL |
| CON | 3.09±0.60 | 8.22±0.91 | 2.71±0.37 |
| CAF | 1.25±0.22 * | 3.33±0.70 * | 2.31±0.43 |
| $CAF_{CR}$ | 1.45±0.36 * | 4.37± 0.78 * | 1.99±0.37 |
| $CAF_{TR}$ | 1.50±0.42 * | 6.85±1.08 † | 2.06±0.22 |
| $CAF_{CRT}$ | 1.52±0.33 * | 6.80±1.12 † | 2.29±0.38 |

Glucose transport was measured at 200 μU.ml$^{-1}$ perfusate insulin concentration as the rate of 2-deoxy-D-2,6[$^3$H]glucose uptake in red gastrocnemius (RG), white gastrocnemius (WG) and soleus (SOL) muscles of perfused rat hindquarters. Values are means±SE and are expressed in μmol.g$^{-1}$.h$^{-1}$ for control rats (CON; $n$=12–14), rats receiving cafeteria diet alone (CAF; $n$=6–7), or supplemented with 2.5% creatine ($CAF_{CR}$; $n$=7), and rats receiving cafeteria diet in conjunction with exercise training alone ($CAF_{TR}$; $n$=6-7) or in combination with creatine supplementation ($CAF_{CRT}$; $n$=6).

* $P$<.05 compared with CON.

† $P$ < .05 compared with CAF.

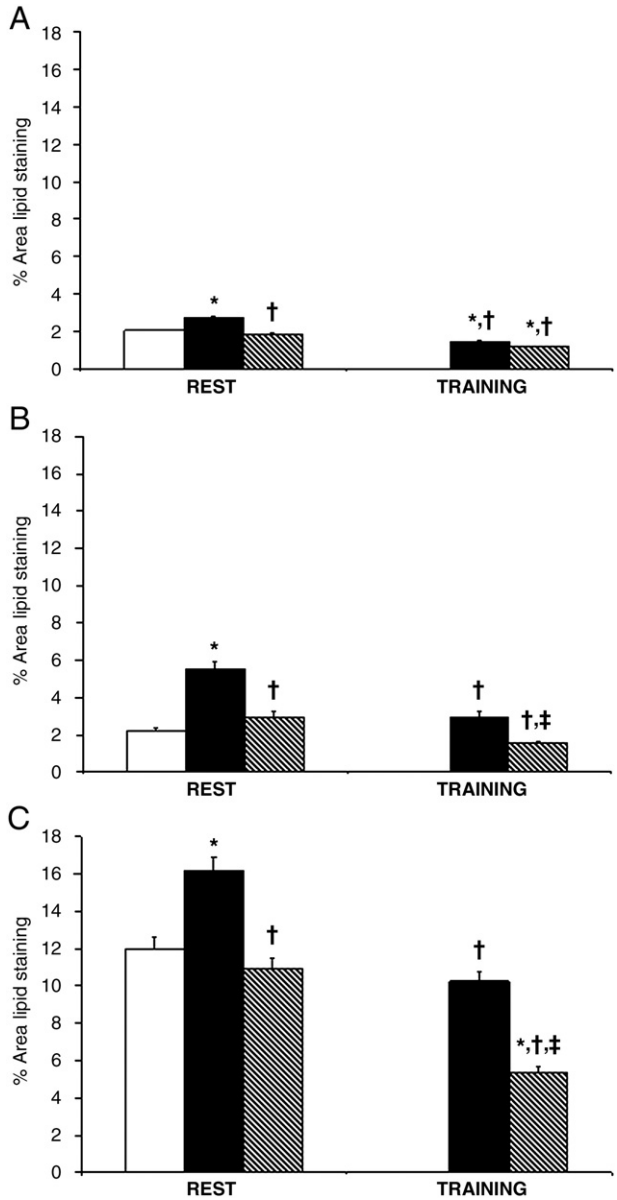


Fig. 1. Effect of cafeteria diet, creatine supplementation and exercise training on intramyocellular lipid content. IMCL content was quantified by fluorescence microscopy on Oil-red-O stained muscle cross-sections in 5 different groups: control rats (CON; $n$=6; white bar), rats receiving cafeteria diet (black bars) alone (CAF; $n$=7) or in the presence of training interventions during the final 4 weeks diet ($CAF_{TR}$; $n$=6) and two groups receiving cafeteria diet supplemented with creatine (striped bars) without ($CAF_{CR}$; $n$=7) or with training interventions in the last 4 weeks of the study ($CAF_{CRT}$; $n$=6). Panels A–C represent IMCL content of Type I fibers of soleus, Type I fibers and Type IIa fibers of EDL, respectively. Values are mean±S.E. and are expressed as percentage of the fiber area covered by fat droplets. *$P$<.05 compared with CON; †$P$<.05 compared with rats fed cafeteria diet; ‡$P$<.05 compared with $CAF_{TR}$.

### 3.4. Intramyocellular lipid content

Fiber-specific IMCL content was measured by ORO-staining in cross sections from m. soleus and EDL (Fig. 1). As expected [29,34,35], in CON, IMCL content was about sixfold higher in Type IIa fibers than

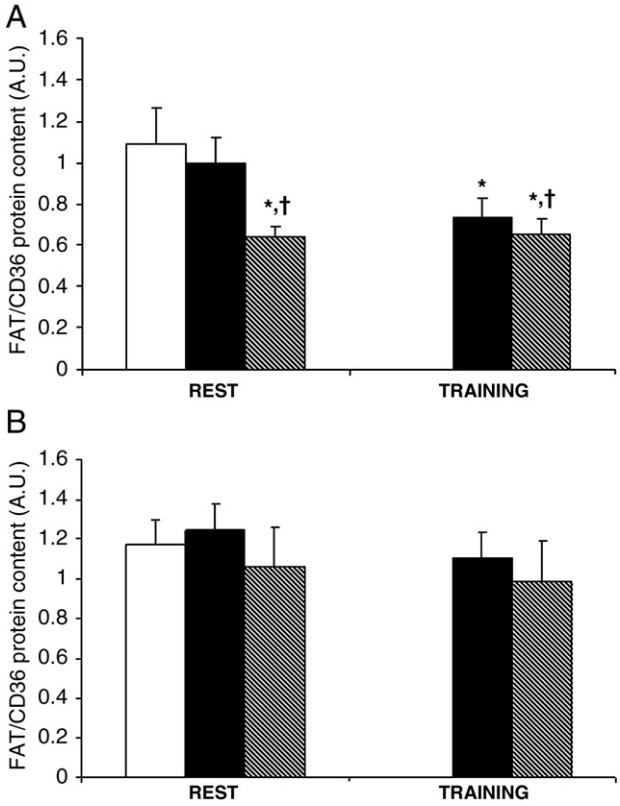


Fig. 2. Effect of cafeteria diet, creatine supplementation and exercise training on FAT/CD36 protein expression in muscle. Protein expression of the free fatty acids translocase FAT/CD36 in soleus (A) and EDL (B) was determined for the control group (CON; $n$=6; white bar), two groups receiving cafeteria diet (black bars) without (CAF; $n$=6) or combined with training ($CAF_{TR}$; $n$=6) and two groups receiving cafeteria diet supplemented with creatine (striped bars) without ($CAF_{CR}$; $n$=6) or combined with training interventions ($CAF_{CRT}$; $n$=6). Values are mean±S.E. and are expressed relative to the pool control (see Materials and Methods). *$P$<.05 compared with control rats; †$P$<.05 compared with rats fed cafeteria diet.

in Type I fibers. Cafeteria diet increased IMCL content in both m. soleus and EDL and the increase was greatest in EDL Type I fibers (+150%, $P$<.05). In m. soleus Type I and in EDL Type IIa fibers, IMCL increased by ~35% ($P$<.01). Feeding rats with cafeteria diet with creatine added did not elevate IMCL. Thus, in $CAF_{CR}$ IMCL content in soleus and EDL Type I and Type IIa fibers was similar to CON. Furthermore, also the training intervention per se prevented cafeteria diet-induced IMCL accumulation. In $CAF_{TR}$ Type I and Type IIa muscle fibers IMCL was consistently lower than CAF ($P$ < .05). In keeping with the observations in $CAF_{CR}$, dietary creatine supplementation stimulated the effect of exercise training to reduce IMCL content. Thus, in $CAF_{CRT}$, IMCL content was ~45% lower than in $CAF_{TR}$ ($P$<.01) in both EDL Type I and IIa fibers. In m. soleus Type I fibers, as well as in EDL Type IIa fibers, IMCL content in $CAF_{CRT}$ was even lower than in CON ($P$<.05).

### 3.5. Muscle FAT/CD36 protein content

The protein content of fatty acid translocase (FAT)/CD36 was assayed by Western blot in both soleus and EDL muscles (Fig. 2). Compared with CON, the administration of cafeteria diet in CAF did not alter FAT/CD36 content in m. soleus. However, in $CAF_{CR}$ FAT/CD36 protein content was about ~36% lower than in either CAF or CON ($P$<.05). Furthermore, compared with CON, exercise training reduced m. soleus FAT/CD36 content by ~35% and ~45% in $CAF_{TR}$ and $CAF_{CRT}$, respectively ($P$<.05). In EDL muscles FAT/CD36 content was affected

Table 5
Effect of cafeteria diet, creatine and training on muscle glycogen, creatine, phosphocreatine and total creatine content

| | | Glycogen | Cr | PCr | Total Cr |
|---|---|---|---|---|---|
| CON | WG | 125.8±7.5 | 49.7±4.1 | 92.9±8.4 | 142.6±9.0 |
| | RG | 130.4±6.3 | 57.9±3.8 | 100.9±7.5 | 151.6±9.5 |
| | SOL | 88.2±8.1 | 45.8±3.5 | 72.5±4.1 | 118.3±4.6 |
| CAF | WG | 143.9±4.6* | 52.6±2.5 | 98.2±4.8 | 150.8±4.6 |
| | RG | 138.2±6.4 | 50.9±2.2 | 93.9±6.7 | 144.7±6.6 |
| | SOL | 97.6±5.8 | 43.5±2.7 | 58.9±4.1* | 101.0±5.1 |
| $CAF_{CR}$ | WG | 146.9±5.7* | 53.5±1.5 | 100.1±7.2 | 153.6±7.0 |
| | RG | 150.2±6.1 | 51.8±3.4 | 91.8±3.8 | 143.6±5.3 |
| | SOL | 101.1±7.6 | 45.1±2.5 | 62.9±4.5 | 107.9±6.0 |
| $CAF_{TR}$ | WG | 138.5±8.3 | 50.2±3.6 | 88.8±3.7 | 138.9±3.7 |
| | RG | 136.8±7.7 | 50.5±5.5 | 101.1±7.1 | 151.6±11.0 |
| | SOL | 103.7±6.9 | 39.7±2.1 | 59.6±5.9 | 99.3±7.0 |
| $CAF_{CRT}$ | WG | 124.1±7.4† | 61.5±6.7 | 91.2±6.6 | 152.7±11.3 |
| | RG | 127.7±6.5 | 57.6±5.1 | 87.6±5.2 | 145.2±5.6 |
| | SOL | 90.5±6.9 | 47.6±2.5 | 65.5±5.5 | 113.0±4.9 |

Values are means±S.E. and are expressed in $mmol^{-1}$.kg DW (dry muscle weight) for control rats (CON; $n$=8), rats receiving cafeteria diet alone (CAF; $n$=16), or supplemented with 2.5% creatine ($CAF_{CR}$; $n$=17), and rats receiving cafeteria diet in conjunction with exercise training alone ($CAF_{TR}$; $n$=8) or in combination with creatine supplementation ($CAF_{CRT}$; $n$=8).
*$P$<.05 compared with CON; †$P$<.05 compared with CAF.

neither by the administration of cafeteria diet, creatine administration nor by exercise training.

### 3.6. Muscle free creatine, phosphocreatine, total creatine, and glycogen content

Muscle free creatine, phosphocreatine, total creatine and glycogen content were measured in white and red gastrocnemius muscles as well as in m. soleus (Table 5). As expected, muscle total creatine, free creatine and phosphocreatine content was higher in white and red gastrocnemius than in soleus muscles. However, there was no significant difference between the experimental conditions. Accordingly, muscle-free creatine and phosphocreatine contents were similar between the groups in any muscle type, except for the slightly lower phosphocreatine content in m. soleus in CAF than in CON ($P$=.052). Glycogen content also was ~30–40% higher in gastrocnemius muscles than in m. soleus. Furthermore, in CAF and $CAF_{CR}$, but not in $CAF_{TR}$ and $CAF_{CRT}$, glycogen was slightly higher than in CON ($P$<.05) in white gastrocnemius. However, neither in red

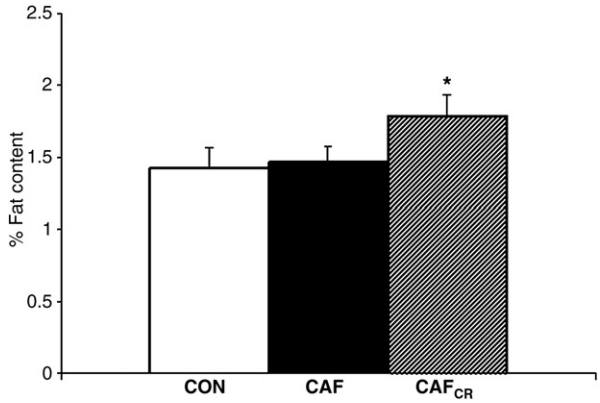


Fig. 3. Effect of cafeteria diet and creatine supplementation on fecal fat content. Fecal fat content was measured in a 3-week supplementary study in the control group (CON; $n$=5; white bar), rats receiving cafeteria diet alone (CAF; $n$=5; black bars) or supplemented with creatine ($CAF_{CR}$; $n$=5; striped bars). Values are mean±S.E. and are expressed as fat percentages of lyophilized fecal mass. *$P$<.05 compared with control rats.

gastrocnemius nor in soleus, glycogen content was significantly different between the experimental groups.

### 3.7. Fecal fat content, liver, and blood triglycerides

To further evaluate the effect of cafeteria diet and creatine administration on whole body fat balance, in a separate 3-week post hoc study we measured fecal fat content in CON, CAF and $CAF_{CR}$. As shown in Fig. 3, the proportion of fecal fat was similar between CON and CAF at ~1.5%. However, in $CAF_{CR}$ compared with CON, fecal fat content increased by ~25% to ~1.8% of fecal mass ($P<.05$). Compared with CON ($7.2\pm0.5$ mmol·l$^{-1}$), the administration of cafeteria diet in CAF caused a 3-fold increase in fasting blood triglyceride concentration ($23.5\pm4.1$ mmol·l$^{-1}$, $P<.05$). However, in $CAF_{CR}$ ($13.9\pm1.5$ mmol·l$^{-1}$), fasting blood triglyceride concentration was markedly reduced compared with CAF ($P < .05$), yet still significantly higher than in CON ($P<.05$). As 90% of fasting blood triglycerides is synthesized in the liver and secreted as components of very low-density lipoprotein particles (VLDL), we also determined liver triglyceride content. Compared with CON [$279.1\pm35.1$ pmol/nmol phospholipids (PPL)] 3 weeks of cafeteria diet significantly increased liver triglyceride content in CAF ($567.6\pm22.9$ pmol/nmol PPL; $P<.05$), but not in $CAF_{CR}$ ($416.1\pm20.4$ pmol/nmol PPL). Moreover, hepatic triglyceride content was lower ($P<.05$) in $CAF_{CR}$ than in CAF.

## 4. Discussion

Over the last decade, a number of studies in humans as well as in rats have provided data to indicate that dietary creatine supplementation may beneficially impact on glucose homeostasis. The current study aimed to investigate the effect of creatine supplementation, alone or in conjunction with exercise training, on glucose tolerance and insulin-stimulated muscle glucose uptake in rats receiving a sucrose-rich cafeteria diet to induce a state of insulin resistance. We show that creatine intake was effective to prevent intramyocellular lipid (IMCL) accumulation during cafeteria diet but failed to beneficially impact on insulin-stimulated muscle glucose transport or whole body insulin sensitivity.

It has been clearly established that mismatching between fatty acid uptake by muscle cells on one side, and mitochondrial fat oxidation on the other side is associated with IMCL accumulation and development of muscular insulin resistance [22,36-39]. In the current study, we used a high-sugar cafeteria diet (see Table 1), instead of a classical experimental 40–50% high-fat diet, to induce insulin resistance. This diet with a more balanced composition better resembles a western diet than the high-fat feeding [40]. Still, despite low fat content, such diet markedly enhances fat delivery to muscles by sucrose-induced stimulation of hepatic lipogenesis, predominantly in the form of increased VLDL synthesis [19,41,42]. Thus, we found cafeteria diet indeed to increase hepatic and circulating fasting blood triglyceride levels already within three weeks. Measurements of the epididymal fat pad mass in a small subgroup of rats indicated that the body weight gain in rats receiving cafeteria diet alone conceivably was largely due to peripheral fat accumulation and that this effect was largely negated by exercise training. More importantly, cafeteria diet besides stimulating peripheral fat accretion, as evidenced by higher epididymal fat mass, also markedly increased IMCL content in both Type I (m. soleus and EDL) and Type IIa (EDL) muscle fibers. Interestingly, the addition of creatine to the cafeteria diet was very potent to preventing diet-induced IMCL accumulation in any muscle type (see Fig. 1). Moreover, in rats performing exercise training during the final stage of the dietary intervention period, creatine intake even caused IMCL to drop below values measured in control rats on a normal diet. Interestingly, recently, we found creatine intake during leg immobilization and retraining in healthy volunteers to result in lower IMCL content in type I fibers of m. vastus lateralis (Vaisy et al., manuscript in preparation). The effect of creatine to prevent IMCL from increasing during cafeteria diet, however, did not rescue insulin sensitivity of muscle glucose transport though (see Table 4). This clearly indicates that intramyocellular triacylglycerol concentration per se is not implicated in modulation of muscular insulin sensitivity, indeed, but probably is just symptomatic for mismatching of muscle free fatty acid uptake and oxidation [37]. Our observation that exercise-induced, but not creatine-induced decrease of IMCL was associated with enhanced insulin-stimulated muscle glucose transport during cafeteria feeding, also supports the opinion that stimulation of IMCL turnover during episodes of dietary lipid challenge, i.e., by muscle contractions, is probably crucial to inhibit accumulation of toxic fatty acid metabolites, such as diacylglycerol and ceramide [37], which in turn may impair insulin signaling [43,44].

Different mechanisms may explain the effect of creatine supplementation to prevent IMCL accumulation during cafeteria diet. In fact, we first wanted to exclude regulation at the site of gastrointestinal lipid absorption. Surprisingly, we found creatine administration to increase fecal fat content by about 25% ($P<.05$, $n=5$), which indicates that creatine by some unknown mechanism interfered with one or more steps of gastrointestinal lipid digestion and/or absorption. The digestion and absorption of lipids require hydrolysis of triglycerides with lingual, gastric and pancreatic lipases, followed by the formation of mixed micelles with bile salt and absorption of micelles across the intestinal wall [45,46]. There is evidence from studies in rodents that creatine absorption from the gastrointestinal tract occurs via a process similar to other nutrients and transporters mediating creatine flux through the intestinal wall have been identified [47-49]. However, whether intestinal creatine and lipid absorption interfere is unknown. The suppression of gastrointestinal lipid digestion and absorption during creatine administration conceivably contributed to the suppression of fasting blood triglyceride levels in rats on cafeteria diet. It is important to consider that more than 90% of fasting blood triglycerides is present in the form of VLDL which are synthesized and secreted by the liver. The fatty acids esterified in the liver to form VLDL originate from either de novo lipogenesis in liver cells, or from circulating fatty acids derived from either dietary lipids (triacylglycerol present in remnant chylomicrons) or triacylglycerol lipolysis in peripheral adipocytes. The liver also stores fatty acids in the form of small droplets containing triglycerides, which can be packed into VLDL particles [50]. Rats receiving creatine supplementation in conjunction with cafeteria diet accumulated less liver triglycerides ($P<.05$) in liver, which was at least partly due to inhibition of dietary lipid absorption. However, we can not exclude that creatine also impacted on either hepatic lipogenesis, VLDL synthesis and/or adipose tissue lipolysis. Nevertheless, peripheral lipid accumulation, measured by weighing epididymal fat mass, was significantly ($P=.05$) lower in the rats receiving creatine together with cafeteria diet than in rats receiving cafeteria diet alone but was still higher than in the control group. As creatine is known to increase lean body mass [6,51,52], this mechanism might be partially responsible for the increase in body weight observed in rats receiving creatine supplementation in combination with cafeteria diet. Still, against the face of approximately twofold increased blood triglyceride concentration compared with control group, creatine administration fully prevented diet-induced IMCL accumulation. This indicates that creatine may inhibit fatty acid import and/or stimulate fatty acid oxidation in muscle cells, even in the absence of exercise training. It is believed that sarcolemmal FAT/CD36 plays a pivotal role in regulating the rate of fatty acid import in muscle cells [53] and FAT/CD36 was also shown to reside on mitochondrial membrane where it can play a role in tuning muscular mitochondrial fat oxidation [54,55]. On the other hand, the role and presence of FAT/CD36 on skeletal muscle mitochondrial membranes, at least in resting metabolic state, were

challenged in a recent report [56]. Here we report the novel finding that creatine administration per se was able to significantly reduce muscle total FAT/CD36 content in the soleus, which probably largely reflects down-regulation of sarcolemmal FAT/CD36 protein [56,57]. Creatine reduced FAT/CD36 content in m. soleus as potently as exercise, and this at least partly explains the action of either intervention to prevent cafeteria diet-induced IMCL accumulation. Still, creatine plus exercise was more effective than creatine alone to inhibit IMCL accumulation, which may be explained by the fact that exercise, but not creatine administration, conceivably stimulated the capacity for fat oxidation [58]. In contrast, despite a marked reduction in IMCL content neither creatine intake nor exercise training affected FAT/CD36 content in EDL muscle. This implies that creatine supplementation and training elicit divergent responses in different muscles. It is therefore reasonable to assume that the decreased IMCL content in m. EDL is mainly due to impaired gastrointestinal lipid absorption.

In this study, exercise training during the final 4 weeks of the cafeteria diet did not alter total muscle FAT/CD36 content. Literature data with regard to the effects of endurance training on total FAT/CD36 expression in muscle are equivocal, with some studies showing increased [59,60] versus others reporting unchanged [61,62] gene and/or protein expression after training. Our current findings are in line with a recent study in Zucker diabetic rats showing that both exercise and metformin, alone or in combination, attenuated the increases in FAT/CD36 content in red gastrocnemius due to 8 weeks of high-fat diet. Still, only in the presence of exercise, these metabolic effects were associated with improved insulin-stimulated muscle glucose transport [57]. Our current and earlier findings [57] taken together thus seem to indicate that exercise is a key-factor for beneficial adaptation of fat metabolism in muscle cells to result in improved insulin action. Support for such contention comes from literature data indicating that exercise is required to localize FAT/CD36 at the mitochondrial membrane, and that this trigger is critical for the maintenance of muscular insulin sensitivity in conditions of diet-induced insulin resistance [58], as in the present study.

There is some anecdotal evidence from our [11,15,16] and other studies [12,13,17,63] that dietary creatine supplementation, alone [14,15,63] or in combination with exercise training [11,12,16,17], may yield beneficial effects on glucose metabolism and insulin action. Therefore, we postulated that creatine supplementation might negate cafeteria diet-induced insulin resistance of glucose uptake. As discussed above, administration of cafeteria diet for 12 weeks caused insulin resistance at whole body level and at the site of skeletal muscle glucose transport, and only 4 weeks of exercise training during the final stage of the dietary intervention period were needed to offset insulin resistance. We have reported elsewhere that these effects were associated with down-regulation (cafeteria diet) versus up-regulation (exercise) of Akt phosphorylation, against the background of normal PI3 kinase activity [26]. This indicates that the mechanism by which cafeteria diet produces insulin resistance is probably different from insulin resistance produced by a typical hypercaloric high-fat diet. However, in contrast with exercise, creatine supplementation did not rescue insulin-stimulated muscle glucose transport (see Table 4). Therefore, our current findings together with earlier observations [64,65] support the opinion that dietary creatine supplementation is not an effective intervention to prevent the development of muscular insulin resistance. Previous studies have demonstrated that creatine administration may increase GLUT4 content and/or glycogen content in both human [11,16,66] and rat [14,15] muscles, but evidence for enhanced insulin action at the site of skeletal muscles due to creatine supplementation is entirely lacking. It could be argued that creatine supplementation failed to impact on muscular insulin sensitivity, because the addition of creatine to cafeteria diet failed to increase muscle creatine content (see Table 5). This finding is compatible with our earlier observation that acute high-dose creatine supplementation, but not longer creatine administration, increased total creatine content in rat and human muscles [2,14,15,66]. Nonetheless, it is important to note that elevated muscle creatine is not a prerequisite for creatine supplementation to generate physiological adaptations at the site of muscle cells [16,66]. Accordingly, here the addition of creatine was successful in counteracting IMCL accumulation during cafeteria diet, against the background of normal muscle creatine content. This indicates that mechanisms other than elevated muscle creatine content *per se* are implicated in generating the physiological adaptations in muscle cells produced by dietary creatine supplementation.

In conclusion, this study for the first time demonstrates that creatine supplementation can inhibit IMCL accumulation due to sucrose-rich cafeteria diet in rats. This effect is at least partly explained by impaired gastrointestinal lipid absorption, as well as by lower muscle FAT/CD36 content, which may result in lower rate of fatty acid import into muscle cells. Furthermore, we here also clearly demonstrate that exercise-induced, but not creatine-induced reduction of IMCL, is associated with improved insulin action on glucose transport in muscle cells.

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## References

[1] Harris RC, Soderlund K, Hultman E. Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. Clin Sci (Lond) 1992;83:367–74.
[2] Vandenberghe K, Goris M, Van Hecke P, Van Leemputte M, Vangerven L, Hespel P. Long-term creatine intake is beneficial to muscle performance during resistance training. J Appl Physiol 1997;83:2055–63.
[3] Hespel P, Eijnde BO, Van Leemputte M, Urso B, Greenhaff PL, Labarque V, et al. Oral creatine supplementation facilitates the rehabilitation of disuse atrophy and alters the expression of muscle myogenic factors in humans. J Physiol 2001;536:625–33.
[4] Hespel P, Derave W. Ergogenic effects of creatine in sports and rehabilitation. Subcell Biochem 2007;46:245–59.
[5] Deldicque L, Louis M, Theisen D, Nielens H, Dehoux M, Thissen JP, et al. Increased IGF mRNA in human skeletal muscle after creatine supplementation. Med Sci Sports Exerc 2005;37:731–6.
[6] Olsen S, Aagaard P, Kadi F, Tufekovic G, Verney J, Olesen JL, et al. Creatine supplementation augments the increase in satellite cell and myonuclei number in human skeletal muscle induced by strength training. J Physiol 2006;573:525–34.
[7] Eijnde BO, Van Leemputte M, Goris M, Labarque V, Taes Y, Verbessem P, et al. Effects of creatine supplementation and exercise training on fitness in men 55-75 yr old. J Appl Physiol 2003;95:818–28.
[8] Tarnopolsky MA. Clinical use of creatine in neuromuscular and neurometabolic disorders. Subcell Biochem 2007;46:183–204.
[9] Gualano B, Artioli GG, Poortmans JR, Lancha Junior AH. Exploring the therapeutic role of creatine supplementation. Amino Acids 2009;38:31–44.
[10] Alsever RN, Georg RH, Sussman KE. Stimulation of insulin secretion by guanidinoacetic acid and other guanidine derivatives. Endocrinology 1970;86:332–6.
[11] Derave W, Eijnde BO, Verbessem P, Ramaekers M, Van Leemputte M, Richter EA, et al. Combined creatine and protein supplementation in conjunction with resistance training promotes muscle GLUT-4 content and glucose tolerance in humans. J Appl Physiol 2003;94:1910–6.
[12] Robinson TM, Sewell DA, Hultman E, Greenhaff PL. Role of submaximal exercise in promoting creatine and glycogen accumulation in human skeletal muscle. J Appl Physiol 1999;87:598–604.
[13] Nelson AG, Arnall DA, Kokkonen J, Day R, Evans J. Muscle glycogen supercompensation is enhanced by prior creatine supplementation. Med Sci Sports Exerc 2001;33:1096–100.

[14] Eijnde BO, Richter EA, Henquin JC, Kiens B, Hespel P. Effect of creatine supplementation on creatine and glycogen content in rat skeletal muscle. Acta Physiol Scand 2001;171:169–76.
[15] Eijnde BO, Jijakli H, Hespel P, Malaisse WJ. Creatine supplementation increases soleus muscle creatine content and lowers the insulinogenic index in an animal model of inherited type 2 diabetes. Int J Mol Med 2006;17:1077–84.
[16] Eijnde BO, Urso B, Richter EA, Greenhaff PL, Hespel P. Effect of oral creatine supplementation on human muscle GLUT4 protein content after immobilization. Diabetes 2001;50:18–23.
[17] Gualano B, Novaes RB, Artioli GG, Freire TO, Coelho DF, Scagliusi FB, et al. Effects of creatine supplementation on glucose tolerance and insulin sensitivity in sedentary healthy males undergoing aerobic training. Amino Acids 2008;34: 245–50.
[18] Holemans K, Caluwaerts S, Poston L, Van Assche FA. Diet-induced obesity in the rat: a model for gestational diabetes mellitus. Am J Obstet Gynecol 2004;190: 858–65.
[19] Petry CJ, Ozanne SE, Wang CL, Hales CN. Early protein restriction and obesity independently induce hypertension in 1-year-old rats. Clin Sci (Lond) 1997;93: 147–52.
[20] Carmona A, Freedland RA. Comparison among the lipogenic potential of various substrates in rat hepatocytes: the differential effects of fructose-containing diets on hepatic lipogenesis. J Nutr 1989;119:1304–10.
[21] Kuhlmann J, Neumann-Haefelin C, Belz U, Kalisch J, Juretschke HP, Stein M, et al. Intramyocellular lipid and insulin resistance: a longitudinal in vivo 1H-spectroscopic study in Zucker diabetic fatty rats. Diabetes 2003;52:138–44.
[22] Bachmann OP, Dahl DB, Brechtel K, Machann J, Haap M, Maier T, et al. Effects of intravenous and dietary lipid challenge on intramyocellular lipid content and the relation with insulin sensitivity in humans. Diabetes 2001;50:2579–84.
[23] Jacob S, Machann J, Rett K, Brechtel K, Volk A, Renn W, et al. Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of Type 2 diabetic subjects. Diabetes 1999;48:1113–9.
[24] Perseghin G, Scifo P, De Cobelli F, Pagliato E, Battezzati A, Arcelloni C, et al. Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a 1H-13C nuclear magnetic resonance spectroscopy assessment in offspring of Type 2 diabetic parents. Diabetes 1999;48:1600–6.
[25] Vaisy M, Szlufcik K, Maris M, De Bock K, Hesselink MK, Eijnde BO, et al. Hyperglycemic diet and training alter insulin sensitivity, intramyocellular lipid content but not UCP3 protein expression in rat skeletal muscles. Int J Mol Med 2010;25:905–13.
[26] Brandt N, De Bock K, Richter EA, Hespel P. Cafeteria diet-induced insulin resistance is not associated with decreased insulin signaling or AMPK activity and is alleviated by physical training in rats. Am J Physiol Endocrinol Metab 2010;299:E215–24.
[27] Ruderman NB, Houghton CR, Hems R. Evaluation of the isolated perfused rat hindquarter for the study of muscle metabolism. Biochem J 1971;124:639–51.
[28] Wojtaszewski JF, Hansen BF, Urso B, Richter EA. Wortmannin inhibits both insulin-and contraction-stimulated glucose uptake and transport in rat skeletal muscle. J Appl Physiol 1996;81:1501–9.
[29] De Bock K, Richter EA, Russell AP, Eijnde BO, Derave W, Ramaekers M, et al. Exercise in the fasted state facilitates fibre type-specific intramyocellular lipid breakdown and stimulates glycogen resynthesis in humans. J Physiol 2005;564: 649–60.
[30] Harris RC, Hultman E, Nordesjo LO. Glycogen, glycolytic intermediates and high-energy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. Methods and variance of values. Scand J Clin Lab Invest 1974;33:109–20.
[31] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911–7.
[32] Van Veldhoven PP, Mannaerts GP. Inorganic and organic phosphate measurements in the nanomolar range. Anal Biochem 1987;161:45–8.
[33] Van Veldhoven PP, Swinnen JV, Esquenet M, Verhoeven G. Lipase-based quantitation of triacylglycerols in cellular lipid extracts: requirement for presence of detergent and prior separation by thin-layer chromatography. Lipids 1997;32: 1297–300.
[34] Hamalainen N, Pette D. The histochemical profiles of fast fiber Types IIB, IID, and IIA in skeletal muscles of mouse, rat, and rabbit. J Histochem Cytochem 1993;41: 733–43.
[35] Kugelberg E. Histochemical composition, contraction speed and fatiguability of rat soleus motor units. J Neurol Sci 1973;20:177–98.
[36] Kraegen EW, Cooney GJ. Free fatty acids and skeletal muscle insulin resistance. Curr Opin Lipidol 2008;19:235–41.
[37] Moro C, Bajpeyi S, Smith SR. Determinants of intramyocellular triglyceride turnover: Implications for insulin sensitivity. Am J Physiol Endocrinol Metab 2007:E203–13.
[38] Straczkowski M, Kowalska I, Dzienis-Straczowska S, Kinalski M, Gorski J, Kinalska I. The effect of exercise training on glucose tolerance and skeletal muscle triacylglycerol content in rats fed with a high-fat diet. Diabetes Metab 2001;27: 19–23.
[39] Bruce CR, Hoy AJ, Turner N, Watt MJ, Allen TL, Carpenter K, et al. Overexpression of carnitine palmitoyltransferase-1 in skeletal muscle is sufficient to enhance fatty acid oxidation and improve high-fat diet-induced insulin resistance. Diabetes 2009;58:550–8.
[40] Muir JG, Walker KZ, Kaimakamis MA, Cameron MA, Govers MJ, Lu ZX, et al. Modulation of fecal markers relevant to colon cancer risk: a high-starch Chinese diet did not generate expected beneficial changes relative to a Western-type diet. Am J Clin Nutr 1998;68:372–9.
[41] Strobl W, Gorder NL, Fienup GA, Lin-Lee YC, Gotto Jr AM, Patsch W. Effect of sucrose diet on apolipoprotein biosynthesis in rat liver. Increase in apolipoprotein E gene transcription. J Biol Chem 1989;264:1190–4.
[42] Chen YD, Coulston AM, Zhou MY, Hollenbeck CB, Reaven GM. Why do low-fat high-carbohydrate diets accentuate postprandial lipemia in patients with NIDDM? Diabetes Care 1995;18:10–6.
[43] Summers SA. Ceramides in insulin resistance and lipotoxicity. Prog Lipid Res 2006;45:42–72.
[44] Timmers S, Schrauwen P, de VJ. Muscular diacylglycerol metabolism and insulin resistance. Physiol Behav 2008;94:242–51.
[45] Iqbal J, Hussain MM. Intestinal lipid absorption. Am J Physiol Endocrinol Metab 2009;296:E1183–94.
[46] Mu H, Hoy CE. The digestion of dietary triacylglycerols. Prog Lipid Res 2004;43: 105–33.
[47] Tosco M, Faelli A, Sironi C, Gastaldi G, Orsenigo MN. A creatine transporter is operative at the brush border level of the rat jejunal enterocyte. J Membr Biol 2004;202:85–95.
[48] Peral MJ, Galvez M, Soria ML, Ilundain AA. Developmental decrease in rat small intestinal creatine uptake. Mech Ageing Dev 2005;126:523–30.
[49] Orsenigo MN, Faelli A, De Biasi S, Sironi C, Laforenza U, Paulmichl M, et al. Jejunal creatine absorption: what is the role of the basolateral membrane? J Membr Biol 2005;207:183–95.
[50] van Herpen NA, Schrauwen-Hinderling VB. Lipid accumulation in non-adipose tissue and lipotoxicity. Physiol Behav 2008;94:231–41.
[51] Balsom PD, Harridge SD, Soderlund K, Sjodin B, Ekblom B. Creatine supplementation per se does not enhance endurance exercise performance. Acta Physiol Scand 1993;149:521–3.
[52] Deldicque L, Theisen D, Bertrand L, Hespel P, Hue L, Francaux M. Creatine enhances differentiation of myogenic C2C12 cells by activating both p38 and Akt/PKB pathways. Am J Physiol Cell Physiol 2007;293:C1263–71.
[53] Bonen A, Chabowski A, Luiken JJ, Glatz JF. Is membrane transport of FFA mediated by lipid, protein, or both? Mechanisms and regulation of protein-mediated cellular fatty acid uptake: molecular, biochemical, and physiological evidence. Physiology (Bethesda ) 2007;22:15–29.
[54] Campbell SE, Tandon NN, Woldegiorgis G, Luiken JJ, Glatz JF, Bonen A. A novel function for fatty acid translocase (FAT)/CD36: involvement in long chain fatty acid transfer into the mitochondria. J Biol Chem 2004;279:36235–41.
[55] Holloway GP, Jain SS, Bezaire V, Han XX, Glatz JF, Luiken JJ, et al. FAT/CD36-null mice reveal that mitochondrial FAT/CD36 is required to upregulate mitochondrial fatty acid oxidation in contracting muscle. Am J Physiol Regul Integr Comp Physiol 2009;297:R960–7.
[56] Jeppesen J, Mogensen M, Prats C, Sahlin K, Madsen K, Kiens B. FAT/CD36 is localized in sarcolemma and in vesicle-like structures in subsarcolemma regions but not in mitochondria. J Lipid Res 2010;51:1504–12.
[57] Smith AC, Mullen KL, Junkin KA, Nickerson J, Chabowski A, Bonen A, et al. Metformin and exercise reduce muscle FAT/CD36 and lipid accumulation and blunt the progression of high-fat diet-induced hyperglycemia. Am J Physiol Endocrinol Metab 2007;293:E172–81.
[58] Schenk S, Horowitz JF. Coimmunoprecipitation of FAT/CD36 and CPT I in skeletal muscle increases proportionally with fat oxidation after endurance exercise training. Am J Physiol Endocrinol Metab 2006;291:E254–60.
[59] Bonen A, Dyck DJ, Ibrahimi A, Abumrad NA. Muscle contractile activity increases fatty acid metabolism and transport and FAT/CD36. Am J Physiol 1999;276: E642–9.
[60] Tunstall RJ, Mehan KA, Wadley GD, Collier GR, Bonen A, Hargreaves M, et al. Exercise training increases lipid metabolism gene expression in human skeletal muscle. Am J Physiol Endocrinol Metab 2002;283:E66–72.
[61] Arkinstall MJ, Tunstall RJ, Cameron-Smith D, Hawley JA. Regulation of metabolic genes in human skeletal muscle by short-term exercise and diet manipulation. Am J Physiol Endocrinol Metab 2004;287:E25–31.
[62] Bruce CR, Anderson MJ, Carey AL, Newman DG, Bonen A, Kriketos AD, et al. Muscle oxidative capacity is a better predictor of insulin sensitivity than lipid status. J Clin Endocrinol Metab 2003;88:5444–51.
[63] Ferrante RJ, Andreassen OA, Jenkins BG, Dedeoglu A, Kuemmerle S, Kubilus JK, et al. Neuroprotective effects of creatine in a transgenic mouse model of Huntington's disease. J Neurosci 2000;20:4389–97.
[64] Newman JE, Hargreaves M, Garnham A, Snow RJ. Effect of creatine ingestion on glucose tolerance and insulin sensitivity in men. Med Sci Sports Exerc 2003;35: 69–74.
[65] Rooney K, Bryson J, Phuyal J, Denyer G, Caterson I, Thompson C. Creatine supplementation alters insulin secretion and glucose homeostasis in vivo. Metabolism 2002;51:518–22.
[66] VAN Loon LJ, Murphy R, Oosterlaar AM, Cameron-Smith D, Hargreaves M, Wagenmakers AJ, et al. Creatine supplementation increases glycogen storage but not GLUT-4 expression in human skeletal muscle. Clin Sci (Lond) 2004;106: 99–106.