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**The anti-diabetic AMPK activator AICAR reduces IL-6 and IL-8 in human adipose tissue and skeletal muscle cells.**

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**ABSTRACT**

Increased production of inflammatory cytokines is suggested to be of importance for initiation and progression of insulin resistance. Chronic treatment with the AMP analogue 5-Aminoimidazole-4-carboxamide-1- $\beta$ -D-ribose (AICAR) has been shown to improve insulin sensitivity and prevent the development of diabetes in rodents. We investigated the effects of AICAR on the expression and production of inflammatory cytokines from human adipose tissue and skeletal muscle cells as well as intact rat skeletal muscles *in vitro*.

AICAR dose-dependently decreased IL-6 gene expression and secretion in human adipose tissue and in human skeletal muscle cells. In parallel, AICAR inhibited IL-8 secretion in human adipose tissue and skeletal muscle cells, as well as reducing IL-8 gene expression in skeletal muscle cells. In intact rat EDL muscle fibres AICAR markedly decreased IL-6 and IL-8 gene expression.

In conclusion, AICAR inhibits the production of IL-6 and IL-8 human adipose tissue and in skeletal muscle cells. We suggest that decreased cytokine production might play a role for the AICAR-induced increase in insulin sensitivity.

## INTRODUCTION

A state of mild chronic sub-clinical inflammation has been associated with features of the metabolic syndrome, including obesity and insulin resistance. In the last decade slightly elevated levels of acute phase reactants such as C-reactive protein (CRP) and elevated levels of various pro-inflammatory cytokines have repeatedly been found in studies on various states of obesity, insulin resistance, and type 2 diabetes (Yudkin et al., 1999; Pradhan et al., 2001). We and others have previously demonstrated that adipocytes and adipose tissue derived macrophages release a variety of pro-inflammatory cytokines and chemokines including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6), and interleukin-8 (IL-8) (Bruun et al., 2001; Ahima and Flier, 2000; Mohamed-Ali et al., 1997). Moreover, production of these adipokines is enhanced in the obese state (Cottam et al., 2004; Kim et al., 2006). Thus, excessive release of pro-inflammatory molecules originating from adipose tissue might play an important role in the pathogenesis of obesity-related insulin resistance by both autocrine/paracrine and endocrine effects (Festa et al., 2000; Berg and Scherer, 2005; de and Olefsky, 2008). Interestingly, pro-inflammatory cytokines especially IL-6 are also secreted by other insulin target tissues including skeletal muscle (Bartoccioni et al., 1994; Hiscock et al., 2004). However, data from human muscle exercise studies have indicated that IL-6 produced by skeletal muscles might improve insulin sensitivity rather than induce insulin resistance (Helge et al., 2003). Exercise is known to induce glucose uptake in skeletal muscle (Ebeling et al., 1993; Kristiansen et al., 2000). Furthermore, in response to exercise the release of IL-6 from working muscles is enhanced (Steensberg et al., 2000). Therefore, it has been suggested that IL-6 plays an important role for the increased glucose uptake during exercise and hence improved insulin sensitivity (Helge et al., 2003). During exercise the fuel-sensing enzyme 5'AMP-activated protein kinase (AMPK) in skeletal muscle is stimulated, and this enzymatic pathway is suggested to be involved in the up-regulation of the glucose transport across the plasma membrane via increased glucose transport protein 4 (GLUT4) content in skeletal muscle (Hayashi et al., 1998; Kurth-Kraczek et al., 1999; Jessen et al., 2003). Accumulating data indicate that stimulation of the AMPK system by the stable AMP analog (5-Aminoimidazole-4-carboxamide-1- $\beta$ -D-ribose; AICAR) or by metformin correspondingly results in increased glucose uptake and increased insulin sensitivity both *in vivo* as well as *in vitro* (Buhl et al., 2001; Merrill et al., 1997; Cuthbertson et al., 2007; Hawley et al., 2002; Zhou et al., 2001).

Chronic treatment of obese rodents with AICAR has been shown to improve insulin sensitivity, reduce blood pressure, improve lipid profiles, and prevent the development of diabetes (Buhl et al., 2002; Pold et al., 2005; Yu et al., 2004). Thus, stimulation of the AMPK by AICAR seems to ameliorate several of the clinical features of the metabolic syndrome. However, the exact pathways by which AICAR affects the metabolic syndrome are yet to be determined. Cytokines produced from the adipose tissue as well as from skeletal muscle might be important mediators and therefore we investigated the effects of AMPK activation (using AICAR) on the expression and production of these cytokines from human adipose tissue and skeletal muscle cells as well as intact rat skeletal muscles *in vitro*.

## RESEARCH DESIGN AND METHODS

**Subjects.** Subcutaneous adipose tissue was obtained from a total of 10 (8 women, 2 men) normal to slightly over-weight subjects (age  $37.5 \pm 3.7$  yr, BMI  $23.8 \pm 0.8$  kg/m<sup>2</sup>). The actual number of subjects in each experiment is indicated in the figure legend. The subjects were all Caucasian, healthy, and did not receive any medication known to influence adipose tissue metabolism. After an overnight fast, subcutaneous adipose tissue from the femoral, gluteal, or abdominal region was obtained by liposuction performed at a cosmetic surgery clinic. The adipose tissue was mixed with isotonic saline and transported to the laboratory in a sterile container within 30 min after removal. The study was approved by the local ethics committee, and all subjects gave written informed consent.

**Animals.** All animal experiments were approved by the Danish Animal Experiments Inspectorate and conducted in conformity with the European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes. Young male Wistar rats weighing approximately 90 g (Møllegaard Breeding Center (Taconic M&B Ltd.), L1. Skensved, DK) were housed in a temperature (21°C) and light-controlled (12:12-h light:dark-cycle) room. They were deprived of food overnight prior to experiments but left unlimited access to water.

**Adipose tissue incubations.** The adipose tissue was transported to the laboratory within 30 minutes after removal. All subsequent procedures were carried out under a laminar airflow hood and under sterile conditions. The tissue was placed in Medium 199, minced into fragments of less than 10 mg each, rinsed in NaCl and incubated as previously described (Kristensen et al., 1999). In brief, 500 mg of adipose tissue fragments were placed in 50 ml plastic tubes containing 5 ml serum-free Medium 199 without phenol red, supplemented with 25 mM HEPES, 1% bovine serum albumin, 1 nM insulin, antipain and leupeptin 20 µl/100 ml and penicillin and streptomycin (10 000 IU) 1 ml/100 ml. The cultures were placed in a humidified incubator at 37°C with a 5 % CO<sub>2</sub> atmosphere and pre-incubated for 24 hours. The medium was then replaced with incubation medium containing IL-1β (2 ng/ml) and AICAR at concentrations as indicated and all cultures were performed as duplicates. After culturing for the time indicated the medium was harvested and kept at -20°C for later cytokine protein measurements. The adipose tissue fragments were immediately frozen in liquid nitrogen and stored at -80°C until RNA isolation.

**Human skeletal muscle cell line in culture.** The human skeletal muscle cell line (SkMC) derived from the rectus abdominis muscle, was purchased from PromoCell bioscience alive GmbH, Heidelberg, Germany. The cells were cultured according to the manufacturer's instructions. Successfully cultured SkMCs were characterized by uniform and cell typical morphology. Cryopreserved SkMCs were thawed and subcultured. Cell culture was performed in a humidified incubator at 37°C with a 5 % CO<sub>2</sub> atmosphere. After 9 days of culture in growth medium (PromoCell bioscience alive GmbH, Heidelberg, Germany), the medium was replaced with differentiation medium (PromoCell bioscience alive GmbH, Heidelberg, Germany), and after another 5 days the cells were used for investigations. The medium was then replaced with incubation medium containing

IL-1 $\beta$  (2 ng/ml) and concentrations of AICAR as indicated. After culturing for the time indicated the medium was harvested and kept at -20°C for later cytokine protein measurements. The cells were immediately frozen in liquid nitrogen and stored at -80°C until RNA isolation.

**Incubation of intact rat skeletal muscles.** After decapitation the extensor digitorum longus (EDL) muscles were rapidly, but carefully removed avoiding stretching of the muscle fibres. The muscles were immediately incubated in 4 ml Krebs-Henseleit bicarbonate buffer (KHBB) containing 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 25 mM NaHCO<sub>3</sub>, 118.5 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl<sub>2</sub>, 1.2 mM MgSO<sub>4</sub>, pH 7.4 supplemented with 5 mM HEPES, 12 mM mannitol, 8 mM glucose and 0.1 % bovine serum albumin. All incubations were carried out at 30°C and under continuous gassing with 95% O<sub>2</sub>/5% CO<sub>2</sub> in a shaking water-bath. Medium was changed every 30 min and following incubation the muscles were blotted briefly on filter paper, trimmed, frozen in liquid nitrogen, and stored at -80°C.

**Isolation of RNA.** Total RNA was isolated from both skeletal muscles and adipose tissue fragments by using the TriZol reagent (Gibco BRL, Invitrogen Ltd., Taastrup, DK). RNA was quantified by measuring absorbancy at 260 and 280 nm. The integrity of the RNA was checked by visual inspection of the two ribosomal RNA's 18S and 28S on an agarose gel.

**Real-time reverse transcription PCR (RT-PCR) measurement of mRNA expression.** RNA was reverse transcribed with reverse transcriptase and random hexamer primers at 23°C for 10 min, 42°C for 60 min, and 95°C for 10 min according to the manufacturer's instructions (GeneAmp RNA PCR Kit from Perkin Elmer Cetus, Norwalk, CT, USA). Then, PCR-mastermix containing the specific primers, Hot Star Taq DNA polymerase, and SYBR-Green PCR-buffer was added. All samples were determined as duplicates. The primer pairs used are listed in Table 1. Quantification of cytokine mRNA relative to the housekeeping mRNA  $\beta$ -actin was performed by using a SYBR-Green real-time PCR assay and an iCycler PCR machine (Bio-Rad Laboratories Inc., California, USA) as previously described (Lihn et al., 2003).

**Cytokine measurements in culture medium.** Cytokine concentration in the incubation mediums was determined by enzyme-linked immunosorbent assays (ELISA). The assay for IL-6 (DuoSet, R&D Systems, Europe, Abingdon, UK) had an intra-assay coefficient of variation of 5.5% ( $n = 12$ ), and the assay for IL-8 had an intra-assay coefficient of variation of 7.9% ( $n = 12$ ).

**Statistical analysis.** Normal distribution of data was tested by Kolmogorov-Smirnov test. For comparison of differences between adipose tissue incubations a two-tailed paired Students t-test or Wilcoxon signed rank test was used according to the result of the Kolmogorov-Smirnov test. For SkMC and rat EDL muscle incubations a two-tailed Students t-test and Mann-Whitney rank sum test was used depending on normal distribution of the data (Kolmogorov-Smirnov test). Results are presented as means  $\pm$  SEM. In dose-response studies test for a dose trend was performed using one way ANOVA with Holm-Sidak test for comparison with control when normality test was passed, in case the normality test failed Kruskal-Wallis one way ANOVA was used instead. All analysis was performed using the SigmaPlot 9.0 statistical package (Systat Software Inc., London, UK). A  $p$ -value below 0.05 was considered as significant.

## RESULTS

### **Effects of AICAR on cytokine gene expression and protein release in adipose tissue.**

In order to investigate an eventual inhibitory effect of AICAR on adipose tissue cytokine production we firstly stimulated the cytokine production by incubating the adipose tissue fragments with the pro-inflammatory cytokine IL-1 $\beta$ . We have previously shown that IL-1 $\beta$  stimulates cytokine production in human adipose tissue *in vitro* (Bruun et al., 2001; He et al., 2000). Incubating with IL-1 $\beta$  (2 ng/ml) adipose tissue fragments for 24 hours increased significantly the release of IL-6 and IL-8 ( $p < 0.001$ ) (Fig. 1A and B). Under these circumstances adding AICAR dose-dependently decreased the production of IL-6 and IL-8 after 24 h (Fig. 1A and B). Time-course studies showed that the inhibitory effect of AICAR on cytokine release was not significant until after 24 hours (Fig. 2A and B).

IL-1 $\beta$  (2 ng/ml) stimulated gene expression of both IL-6 and IL-8, albeit the increase in mRNA levels did not reach statistical significance (Table 2). Similar to the findings for protein release, adipose tissue IL-6 and IL-8 mRNA expression were dose-dependently decreased by AICAR (Table 2).

### **Effects of AICAR on GLUT4 and adiponectin gene expression in adipose tissue.**

In order to control for a possible general toxic effect of AICAR on the cultured cells we investigated the effects of AICAR on the GLUT4 and adiponectin gene expression. As shown in Fig. 3 AICAR dose-dependently increased GLUT4 mRNA levels in adipose tissue fragments. Incubating with IL-1 $\beta$  (2 ng/ml) did not affect GLUT4 gene expression (Fig. 3 insert). Likewise, adiponectin mRNA levels were increased dose-dependently by AICAR (Fig. 4). Final AICAR concentrations of 0.5 mM, 1 mM, and 2 mM increased adiponectin gene expression significantly (3-4 fold,  $p < 0.05$ ).

### **Effects of AICAR on cytokine gene expression and protein release in human skeletal muscle cells.**

Similar to our findings in human adipose tissue fragments IL-1 $\beta$  had a marked stimulatory effect on IL-6 ( $p < 0.05$ ) and IL-8 ( $p < 0.05$ ) secretion from the SkMC-cells (Fig. 5, A and B). Furthermore, AICAR dose-dependently decreased the secretion of these cytokines (Fig. 5, A and B). The gene expression data closely resembled our findings on the protein level; IL-1 $\beta$  had a marked stimulatory effect on IL-6 and IL-8 mRNA expression ( $p < 0.05$ ), and adding AICAR resulted in a dose-dependent decrease in the IL-8 and IL-6 mRNA expression (Table 3).

### **Effects of AICAR on cytokine production and gene expression in rat EDL muscle.**

To investigate whether the changes observed in the skeletal muscle cell line *in vitro* also was operational in intact skeletal muscle, we studied intact rat EDL-muscle group *in vitro*. After 240 min of incubation skeletal muscle gene expression

of IL-6 and IL-8 was powerfully and highly significantly decreased by 0.5 mM AICAR (Table 4).

## DISCUSSION

Inflammatory cytokines have been linked to insulin resistance (Festa et al., 2000; Berg and Scherer, 2005), and AICAR is known to increase insulin sensitivity *in vivo*. In light of this, it is interesting that we, in the present study, were able to show an AICAR-induced dose-dependent decreased IL-6 and IL-8 production and gene expression in both human adipose tissue fragments as well as in a human skeletal muscle cell line *in vitro*. To our knowledge this is the first study demonstrating that AICAR inhibits the release of IL-6 and IL-8 in human skeletal muscles cells. Concerning the effects of AICAR on cytokine production in adipose tissue, these findings are partly in line with previous findings in human adipocytes in a study by Sell et al. (Sell et al., 2006). They found that AICAR (1 mM) significantly decreased the secretion of IL-6 but not IL-8 from human adipocytes. In our study, we used adipose tissue fragments, consisting of a mixture of adipocytes and non-fat cells. Since the inflammatory cytokines released from whole adipose tissue originate predominantly from the non-fat cells especially the macrophages (Fain et al., 2004) this might explain why we were able to demonstrate an inhibitory effect of AICAR on IL-8 as well as IL-6, in contrast to what was found in isolated adipocytes.

We additionally found that AICAR increases the GLUT4 gene expression in adipose tissue. This indicates that AICAR affects genes differently, and consequently the reduced mRNA level of the cytokines is not caused by a general toxic effect of AICAR. However, still the mechanism by which AICAR decrease cytokine levels warrants further investigations. In glial cells AICAR induced activation of AMPK has been shown to inhibit expression of pro-inflammatory cytokines by attenuation of nuclear factor- $\kappa$ B activation (NF- $\kappa$ B) and nuclear translocation of NF- $\kappa$ B (Giri et al., 2004). Given that AICAR activates AMPK in both adipose tissue (Lihn et al., 2004; Sell et al., 2006) and in skeletal muscle (Merrill et al., 1997; Buhl et al., 2001), activation of AMPK followed by inhibition of the NF- $\kappa$ B pathway might be the major mechanism whereby AICAR affects cytokine gene expression and production.

Other adipokines such as adiponectin may also be involved in the regulation of cytokine production in adipose tissue and skeletal muscle. AICAR induces adiponectin secretion (Sell et al., 2006), and as we show in the present study as well as a previous study (Lihn et al., 2004), AICAR also induces adiponectin gene expression. Furthermore, mutual regulation has been shown for adiponectin and several cytokines especially TNF- $\alpha$  and IL-6 (Dietze-Schroeder et al., 2005; Bruun et al., 2003; Maeda et al., 2001). Therefore, it might be speculated that AICAR-induced changes in adiponectin levels also contribute to the inhibitory effects observed on cytokines. However, the reverse mechanism by which AICAR primarily reduce cytokines levels and secondary to this increase adiponectin levels is also conceivable (Lihn et al., 2004). The question of which is the cause and which the consequence in relation to the observed changes in adiponectin and cytokine levels induced by AICAR needs to be resolved in future investigations.

To our knowledge this is the first study demonstrating an AICAR-induced increase

in GLUT4 gene expression in human adipose tissue. *In vivo* treatment of rodents with AICAR has previously been shown to increase insulin sensitivity and increase GLUT4 content in skeletal muscle (Buhl et al., 2001; Jessen et al., 2003). We were able to elaborate further on this by demonstrating that AICAR also in human adipose tissues may increase the insulin sensitive GLUT4 mRNA expression *in vitro*. This finding adds to the notion of AMPK activation playing a role in relation to increased glucose uptake in insulin sensitive tissues such as skeletal muscle and adipose tissue.

Finally, we studied an intact muscle group in order to clarify if our findings in the skeletal muscle cell line could be extrapolated to intact muscle fibres. In this model, rat EDL muscle was used, and it turned out that the experimental setting with *in vitro* incubation for 240 min was a very strong stimulus for cytokine mRNA expression. The cause of this stimulation is not entirely clear, the media was changed every half hour, and it was constantly oxygenated. Using this model we could demonstrate that AICAR also in intact rat EDL muscles has a marked inhibitory effect on IL-6 and IL-8 mRNA expression.

In conclusion: AICAR inhibits secretion and gene expression of IL-6 and IL-8 from human adipose tissue fragments as well as from cultured human skeletal muscle cells. In intact rat EDL muscle fibres AICAR likewise markedly decreased IL-6 and IL-8 mRNA levels.

Thus, AICAR inhibits production of several inflammatory cytokines in human adipose tissue fragments as well as in human (and rat) skeletal muscle. These cytokines are suggested to play a role in relation to insulin resistance. Therefore, regulation of cytokine production by AICAR in insulin sensitive tissues might play a role in relation to AICAR-induced increase in insulin sensitivity.

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**Duality of interest** The authors declare that there is no duality of interest associated with this manuscript.

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## LEGENDS

Fig. 1. Dose-response effects of AICAR on adipose tissue IL-6 (A) and IL-8 (B) protein concentration in culture medium (ng/ml). Adipose tissue fragments incubated for 24 hours (n = 6) with medium +/- IL-1 $\beta$  (2 ng/ml). Mean values  $\pm$  SEM. ANOVA for linear trend;  $p < 0.001$  (IL-6 and IL-8). \*  $p < 0.05$ , \*\*\*  $p < 0.001$ .

Fig. 2. Time-course effects of AICAR on adipose tissue IL-6 (A) and IL-8 (B) protein concentration in culture medium (ng/ml) (n = 6). Adipose tissue fragments with medium +/- IL-1 $\beta$  (2 ng/ml) and +/- AICAR (0.5 mM). Mean values  $\pm$  SEM. \*  $p < 0.05$ , \*\*  $p < 0.01$ .

Fig. 3. Dose-response effects of AICAR on GLUT4 mRNA expression. Adipose tissue fragments incubated for 24 h, stimulated with IL-1 $\beta$  (2 ng/ml) +/- AICAR (0.5mM) (insert, n = 5), and un-stimulated (n = 5). GLUT4 mRNA levels are expressed relative to  $\beta$ -actin mRNA levels. Mean values  $\pm$  SEM. ANOVA for linear trend;  $p < 0.01$ . \*  $p < 0.05$ .

Fig. 4. Dose-response effects of AICAR on adiponectin mRNA expression. Adipose tissue fragments incubated for 24 h (n = 6). Adiponectin mRNA levels are expressed relative to  $\beta$ -actin mRNA levels. Mean values  $\pm$  SEM. ANOVA for linear trend  $p < 0.001$ . \*  $p < 0.05$ , \*\*  $p < 0.01$ .

Fig. 5. Dose-response effects of AICAR on skeletal muscle cell IL-6 (A) and IL-8 (B) protein concentration in culture medium (ng/ml). Incubation for 48 hours (n = 4) with medium +/- IL-1 $\beta$  (2 ng/ml). Mean values  $\pm$  SEM. ANOVA for linear trend;  $p < 0.001$  (IL-6) and  $p < 0.01$  (IL-8). \*  $p < 0.05$ , \*\*\*  $p < 0.001$ .

Table 1. Primer sequences for RT-PCR.

Table 2. Effects of AICAR on adipose tissue IL-6 and IL-8 gene expression. Cytokine mRNA levels are expressed relative to  $\beta$ -actin. Adipose tissue fragments incubated for 24 hours (n = 3) with medium +/- IL-1 $\beta$  (2 ng/ml). Mean values  $\pm$  SEM. Control compared with IL-1 $\beta$ ; <sup>a</sup>  $p = 0.068$ , <sup>b</sup>  $p = 0.051$ . *P*-value reflects the test result of a two-tailed paired Students t-test comparing Control with IL-1 $\beta$  + AICAR 2 mM; \*  $p < 0.05$ .

Table 3. Effects of AICAR on skeletal muscle cell IL-6 and IL-8 gene expression. Cytokine mRNA levels are expressed relative to  $\beta$ -actin. Incubation for 48 hours (n = 4) with medium +/- IL-1 $\beta$  (2 ng/ml). Mean values  $\pm$  SEM. Control compared with

IL-1 $\beta$ ; <sup>a</sup>  $p = 0.029$ , <sup>b</sup>  $p = 0.029$ .  $P$ -value reflects the test result of a two-tailed paired Students t-test comparing Control with IL-1 $\beta$  + AICAR 2 mM; \*  $p < 0.05$ , \*\*\*  $p < 0.001$ .

Table 4. Effects of AICAR (0.5 mM) on rat EDL muscle IL-6 and IL-8 gene expression. Cytokine mRNA levels are expressed relative to  $\beta$ -actin. EDL muscles incubated for 240 min ( $n = 6$ ). Mean values  $\pm$  SEM. \*\*  $p < 0.01$ .

**Table 1.** Primer sequences for RT-PCR.

Species	Gene	Sense	Anti-sense
Human	IL-6	5'-AAATGCCAGCC- TGCTGACGAAC	5'- AACAACAATCTGA- GGTGCCCATGCTAC
Human	IL-8	5'-AACTTCTCCACAACCCTCTG	5'-TTGGCAGCCTTCCTGATTC
Human	Adiponectin	5'-CATGACCAGG- AAACCACGACT	5'-TGAATGCTGAGCGGTAT
Human and rat	GLUT4	5'-GCACCGCCAGGACATTGTTG	5'- CCCCCTCAGCAGCGAGTGA
Human And rat	$\beta$ -actin	5'-TGTGCCATCTA- CGAGGGGTATGC	5'-GGTACATGGTGG- TGCCGCCAGACA
Rat	IL-6	5'-ATGAAGTTTCT- CTCCGCAAGA	5'-CTCCGGACTTG- TGAAGTAGGG
Rat	IL-8	5'-TGAGACATGAACGGAATCTGC	5'-TTCGCTGGACAAGTGACGG

**Table 2.** Effects of AICAR on adipose tissue IL-6 and IL-8 gene expression.

Gene	Control	IL-1 $\beta$ (2ng/ml)	IL-1 $\beta$ + AICAR 0.5 mM	IL-1 $\beta$ + AICAR 2 mM	<i>p</i> -value
IL-6 mRNA	2.8 $\pm$ 1.4 <sup>a</sup>	10.2 $\pm$ 2.4 <sup>a</sup>	7.3 $\pm$ 2.2	1.4 $\pm$ 0.4	0.048*
IL-8 mRNA	7.4 $\pm$ 1.4 <sup>b</sup>	69.8 $\pm$ 14.8 <sup>b</sup>	85.0 $\pm$ 14.0	36.7 $\pm$ 6.8	0.054

Cytokine mRNA levels are expressed relative to  $\beta$ -actin. Adipose tissue fragments incubated for 24 hours ( $n = 3$ ) with medium +/- IL-1 $\beta$  (2 ng/ml). Mean values  $\pm$  SEM. Control compared with IL-1 $\beta$ ; <sup>a</sup>  $p = 0.068$ , <sup>b</sup>  $p = 0.051$ . *P*-value reflects the test result of a two-tailed paired Students t-test comparing Control with IL-1 $\beta$  + AICAR 2 mM; \*  $p < 0.05$ .

**Table 3.** Effects of AICAR on skeletal muscle cell IL-6 and IL-8 gene expression.

Gene	Control	IL-1 $\beta$ (2ng/ml)	IL-1 $\beta$ + AICAR 0.5 mM	IL-1 $\beta$ + AICAR 2 mM	p-value
IL-6 mRNA	$4.2 \times 10^{-3} \pm 0.6 \times 10^{-3}^a$	$3.1 \pm 0.4^a$	$1.8 \pm 0.4$	$0.22 \pm 0.08$	< 0.001***
IL-8 mRNA	$4.3 \times 10^{-2} \pm 0.8 \times 10^{-2}^b$	$19.2 \pm 0.9^b$	$18.5 \pm 3.4$	$8.5 \pm 3.8$	0.033*

Cytokine mRNA levels are expressed relative to  $\beta$ -actin. Incubation for 48 hours (n = 4) with medium +/- IL-1 $\beta$  (2 ng/ml). Mean values  $\pm$  SEM. Control compared with IL-1 $\beta$ ; <sup>a</sup>p = 0.029, <sup>b</sup>p = 0.029. P-value reflects the test result of a two-tailed paired Students t-test comparing Control with IL-1 $\beta$  + AICAR 2 mM; \* p < 0.05, \*\*\* p < 0.001.

**Table 4.** Effects of AICAR (0.5 mM) on rat EDL muscle IL-6 and IL-8 gene expression.

Gene	Control	AICAR 0.5 mM	<i>p</i> -value
IL-6 mRNA	$32.06 \times 10^{-2} \pm 5.37 \times 10^{-2}$	$4.90 \times 10^{-2} \pm 1.87 \times 10^{-2}$	0.002**
IL-8 mRNA	$41.68 \times 10^{-4} \pm 6.52 \times 10^{-4}$	$3.25 \times 10^{-4} \pm 0.591 \times 10^{-4}$	0.002**

Cytokine mRNA levels are expressed relative to  $\beta$ -actin. EDL muscles incubated for 240 min (n = 6). Mean values  $\pm$  SEM. \*\*  $p < 0.01$ .









