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Adiponectin and leptin actions on DNA synthesis and cell death of porcine myoblasts are dependent on the cellular milieu and related to p44/42 signalling

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Introduction

Investigating the growth mechanisms of skeletal muscle and adipose tissue including their interactions through myokines and adipokines is an important aim in animal science and human medical research. To further elucidate the role of adiponectin and leptin in muscle cell growth, this study was conducted to investigate their effects on cell proliferation and cell death in porcine myoblasts. Moreover, the specific activation of key signalling molecules involved in adiponectin and leptin action was studied.

Hypothesis

The presence of growth factors in the culture medium is an important factor for the adipokine effects on the *in vitro* growth of porcine myoblasts.

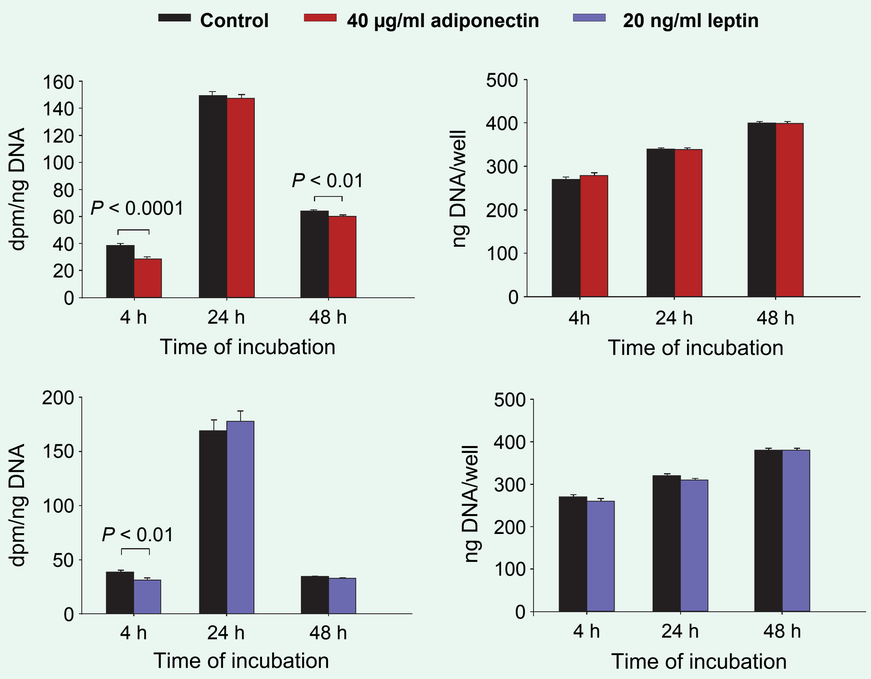
Materials and Methods

Satellite cells from the *semimembranosus* muscles of newborn piglets (German Landrace) were grown in gelatine-coated 96-well plates followed by treatment with adiponectin (40 µg/ml) or leptin (20 ng/ml) in growth factor-supplemented (PDGF-BB, bFGF) serum-free or low-serum medium (1% FBS) for 4, 24 or 48 h. The cultures were analysed for DNA synthesis by ^{3}H-thymidine incorporation. Cell viability was determined by measuring lactate dehydrogenase (LDH) activity in the supernatant. The specific activation of p44/42 MAPK and its downstream targets c-fos and p53 were analyzed by immunoblotting. Statistical analysis of the data was performed by ANOVA using the GLM procedure of SAS (SAS Inst. Inc., USA).

Results

Growth factor-supplemented serum-free conditions

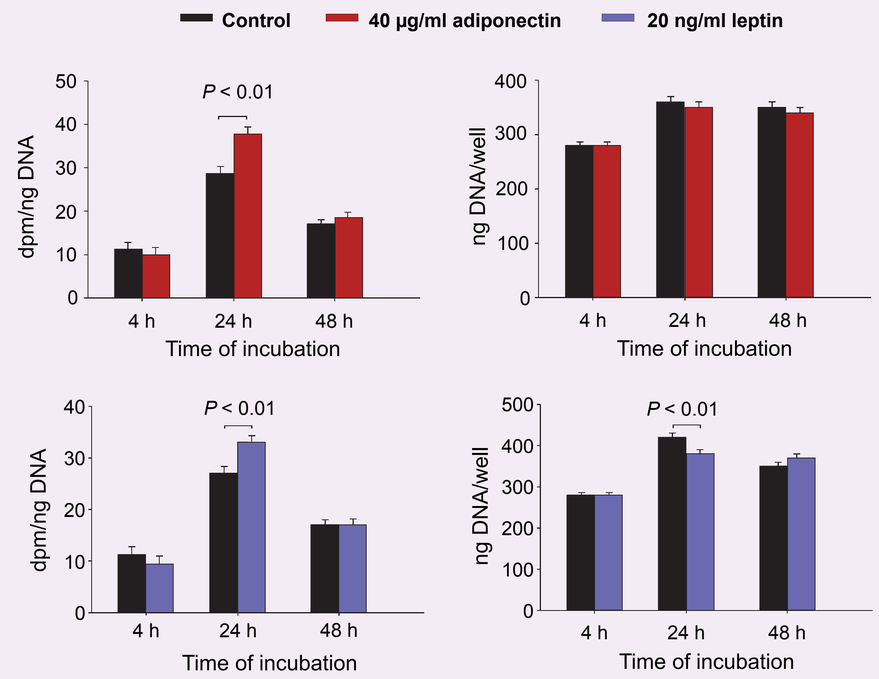
DNA synthesis



Leptin and adiponectin decreased DNA synthesis rate after short-time exposure.

Both adipokines increased DNA synthesis rate associated with decreases in cell number.

Low-serum conditions



Cell Viability

	Treatment	Time of Exposure		SE max
		10 h	24 h	48 h	
LDH/DNA (mIU/µg) (n=10)	Control	0.65	0.46	0.84[a]	0.06
	40 µg/ml Adiponectin	0.66	0.35	0.67[b]	0.06
	20 ng/ml Leptin	0.64	0.34	0.64[b]	0.06

abc: LSMeans within a column not sharing a common letter significantly differ (P<0.05)

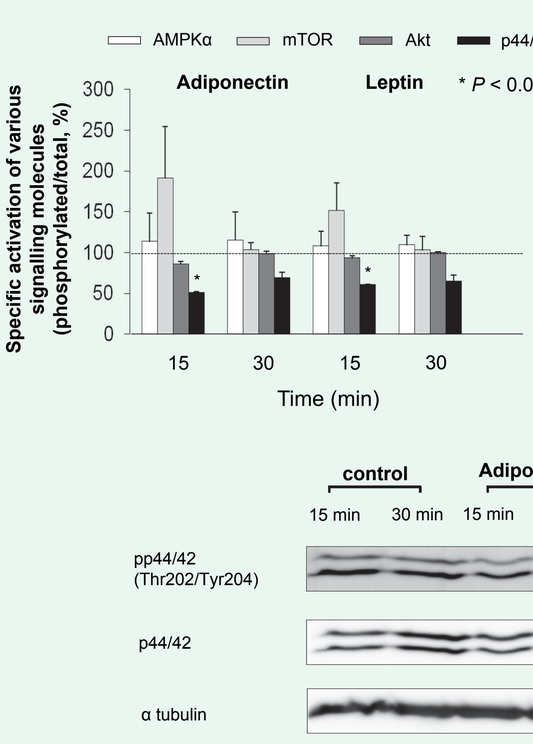
Diminished rate of cell death was observed after adipokine treatment.

Cell viability was reduced by leptin and adiponectin.

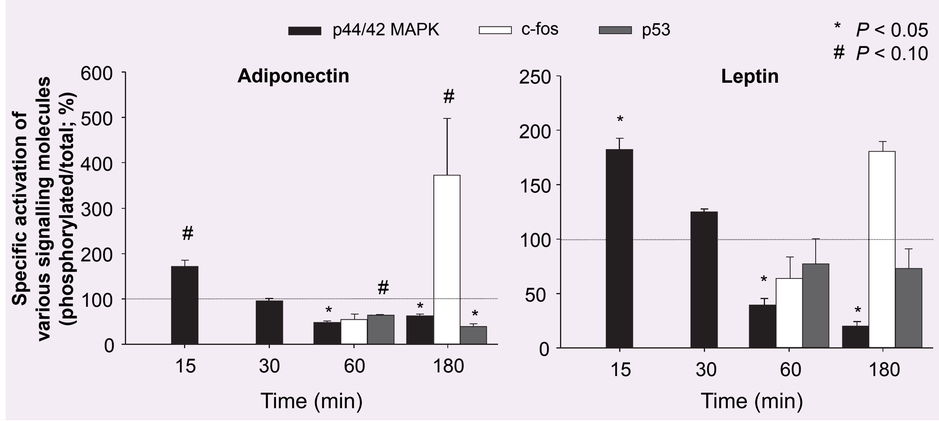
	Treatment	Time of Exposure		SE max
		24 h	48 h	
LDH/DNA (mIU/µg) (n=10)	Control	1.24[a]	3.21	0.16
	40 µg/ml Adiponectin	1.65[b]	3.54	0.16
	20 ng/ml Leptin	1.57[b]	3.45	0.16

abc: LSMeans within a column not sharing a common letter significantly differ (P<0.05)

Signalling



The specific activation of p44/42 MAPK was decreased after 15-min treatment with leptin or adiponectin.



Leptin and adiponectin led to a transient activation of p44/42 MAPK that was even decreased after long-time exposure.

Conclusions

- The influence of leptin and adiponectin on the growth of porcine skeletal muscle cells depends on the particular culture conditions and is related to p44/42 MAPK signalling.
- The action of p44/42 MAPK appears to be too transient to have long-lasting significant effects downstream targets like p53 and c-fos.
- The presence of growth factors in culture medium seems to attenuate adverse effects of the adipokines on myoblast growth.

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