ISSN 1672-9145 **Acta Biochimica et Biophysica Sinica** 2007, **39**(7): 490–498 **CN** 31-1940/Q

Effects of the Monoclonal Antibody against Porcine 40 kDa Adipocyte-specific Plasma Membrane Protein on Adipocytes and Carcass Composition

Shizheng GAO, Changrong GE, Xi ZHANG*, and Yonggang LIU

Key Laboratory of Animal Nutrition and Feed of Yunnan Province, Yunnan Agricultural University, Kunming 650201, China

Abstract The effects of the mouse monoclonal antibody against 40 kDa adipocyte-specific plasma membrane protein on porcine adipocytes and carcass composition were investigated *in vitro* and *in vivo*. Results revealed that the *in vitro* complement-mediated cytotoxicity of this monoclonal antibody can lead to adipocyte lysis, remarkable reduction of adipocyte lipid accumulation ($P<0.01$), and significant decrease of well-differentiated fat cells ($P<0.01$). Treatment of adipocytes with this antibody alone *in vitro* did not induce cell lysis, but could lead to noticeable reduction of well-differentiated cells and lipid accumulation ($P<0.05$) at the pre-adipocyte stage. *In vivo*, pigs injected with 0.5 mg/kg or 1.0 mg/kg of antibody showed smaller adipocyte sizes ($P<0.01$) and reduced lipid accumulation of adipocytes ($P<0.01$). Our results also indicated that pigs intraperitoneally or subcutaneously immunized with 0.5 mg/kg of monoclonal antibody at 15 kg or 1.0 mg/kg antibody at 60 kg had a higher lean meat percentage ($P<0.05$), larger loin eye area ($P<0.05$), lower fat meat percentage ($P<0.05$), less backfat thickness ($P<0.05$) and smaller leaf fat weight ($P<0.05$) than the control pigs, but other carcass traits such as caul fat weight, heart weight, liver weight, spleen weight, kidney weight, lung weight, and dressing percentage were not significantly affected. These results suggested that this monoclonal antibody could be applied to restrain excessive fat deposition in porcine production.

Keywords monoclonal antibody; adipocyte; carcass composition; pig

Excessive fat in domestic animals has been recognized as detrimental to lowering production costs and a health risk to human consumers. Current meat animal production systems produce a large amount of fat in carcasses. Finding effective methods to suppress excessive fat deposition in the production of livestock such as pig is of utmost importance.

Various approaches have been attempted to solve the excessive fat deposition problem. Recently, studies have used polyclonal and monoclonal antibodies to depress the development of adipose tissue and great progress has been achieved. Flint *et al*. first showed that polyclonal antibodies raised in sheep against rat adipocyte plasma membranes were cytotoxic *in vitro* and caused a decrease of the rat body fat *in vivo* [1] . In other studies, polyclonal antibodies raised against fat cells or adipocyte plasma membrane proteins of different animals have been reported [2–6]. These studies revealed that those antibodies could lead to fat cell lysis *in vitro* or *in vivo* in different animals, and therefore decrease body fat considerably. However, these polyclonal antibodies not only specifically act on the fat cells, but also produce serious side-effects to several other tissue cells for cross-reaction. In order to improve the specificity of anti-adipocyte antibodies, a few studies have focused on the production of monoclonal antibodies against fat cell surface proteins for some animals such as pig [7, 8] and chicken [9]. This research indicated that the depressive effect of monoclonal antibodies on adipocytes and pre-adipocytes were revealed in primary porcine and chicken stromal-vascular cultures and this can significantly reduce the fat mass weight of pigs, chickens and rats by

Received: January 6, 2007 Accepted: March 20, 2007

This work was supported by the grants from the Key Project of the Natural Science Foundation of Yunnan Province (No. 2000C005Z) and the National Natural Science Foundation of China (No. 30260079)

*Corresponding author: Tel, 86-871-5227795; Fax, 86-871-5227284; E-mail, zhangxi_km@hotmail.com

DOI: 10.1111/j.1745-7270.2007.00311.x

injection [9,10]. This research suggested that the immunological approach, using the specific monoclonal antibody against porcine adipocyte plasma membrane protein, might be a potential method to suppress excessive fat deposition in the production of pigs.

In our previous experiments, a 40 kDa porcine adipocyte-specific plasma membrane protein had been identified and prepared [11], and subsequently one mouse monoclonal antibody was raised against this 40 kDa protein [12]. This monoclonal antibody showed desirable specificity to subcutaneous fat cells. Western blot identification revealed that the antibody could only bind to the 40 kDa protein of cell membrane fractions in subcutaneous dorsal and abdominal adipose tissue, but not to any protein of cell membrane fractions in mesenteric or parietal adipose tissue, heart, liver, spleen, lung, kidney, skeletal muscular or red blood cells.

This implied that this monoclonal antibody could display cytotoxicity only to the adipocytes from subcutaneous and abdominal adipose tissues and produce a suppressive effect to these fat cells and tissues, but would not produce side-effects to other tissues. In the present experiment, we tested the depressive effects of this monoclonal antibody on adipocytes *in vitro* and on adipose tissues *in vivo* and also tested its side-effects by investigating its effects on other carcass traits. This is necessary for further application of the monoclonal antibody to restrain excessive fat deposition in future porcine production.

Materials and Methods

Cell culture

Landrace×Saba (LS) pigs between 21 and 28 d old were exsanguinated and blood serum was aseptically isolated, then subcutaneous adipose tissue was aseptically sampled, and stromal-vascular cells were isolated and cultured as described previously [10,13], with some modifications. Briefly, adipose tissue (10 g) was minced and digested in a digestion buffer (2 ml/g of tissue) consisting of M199 medium (Gibco BRL, Carlsbad, USA), 2 mg/ml bovine serum albumin and 2 mg/ml collagenase II (800 U/mg; Sigma-Aldrich, St. Louis, USA) in a shaking water bath for 2 h at 37 ºC. After successive filtration through 200, 100 and 25 μm nylon meshes, the cell suspension was counted on a hemocytometer to estimate the concentration of stromal-vascular cells. Cells in 0.5 ml of plating medium were inoculated into 24-well plates (2 cm^2/well) at densities of 1.5×10^4 cells/cm^2. They were maintained in a humidified 5% CO_2 atmosphere at 37 ºC. Plating medium consisted of Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml fungizone, 0.25 μg/ml nystatine, 4 mM L-glutamine, 0.4 mIU/ml insulin, 100 nM cortisol, 20% fetal bovine serum (Gibco) and 5% porcine serum (isolated from the pigs that provided the subcutaneous adipose tissue). Culture media were changed every other day.

Determination of cytotoxicity on adipocytes *in vitro*

The mouse monoclonal antibody prepared by us previously [12] was added into a 3-wells of plate at a concentration of 10 μg/ml together with baby rabbit complement (Gibco) at a dilution of 1:20 on day 5 of culture (the time pre-adipocytes begin to accumulate lipid droplets). Another two treatments of the culture were prepared by adding antibody alone or complement alone. Neither complement nor antibody was added to the control. Cultures were fixed 3 d later in phosphate-buffered saline containing 10% formalin and stained with Oil red O. The triglyceride content of adipocytes in each well was detected through the method described previously by Ramirez-Zacarias *et al*. [14]. The proportion of differentiated adipocytes (Oil red positive) in the total cell population was estimated by direct counting using a microscope (200×; AM311). Twenty-five different areas were counted per culture well.

Determination of effects on adipose *in vivo*

LS pigs (weaned, body weights approximately 15 kg) were obtained from a swine breeding facility in Yunnan province. Eighteen male pigs were randomly assigned to three treatment groups (six pigs per group). These three groups were given intraperitoneal injection of 0, 0.5 or 1.0 mg/kg of purified antibody in 10 ml saline at 15 kg weight.

Pigs were weighed weekly and were killed at 90 kg weight. The subcutaneous dorsal adipose tissue was sampled immediately for frozen section, detection of triglyceride content in adipose tissue and determination of adipocyte size distribution. The frozen section of subcutaneous dorsal adipose tissue was carried out as described by Seveus *et al*. [15]. The triglyceride content of adipocytes was detected through the method described above. Adipocyte size in subcutaneous dorsal adipose tissue was determined as described by de Clercq *et al*. [10].

To further detect the *in vivo* effects of the antibody on carcass composition of pigs, 70 LS pigs (body weights approximately 15 kg, weaned) had been obtained from Chuxiong pig breeding farm in Yunnan province. These pigs were randomly assigned to 14 treatment groups (five

pigs per group). Intraperitoneal injections were given to pigs in groups 1–6. Pigs in groups 1–3 (15 kg) were injected with 0.1, 0.5 and 1.0 mg/kg, repectively, of purified monoclonal antibody in saline. Pigs in groups 4–6 were injected with 0.1, 0.5 and 1.0 mg/kg, respectively, of purified antibody in the same volume of saline when they were 60 kg. Subcutaneous injections were given to pigs in groups 7–12. Pigs in groups 7–9 were immunized with 0.1, 0.5 or 1.0 mg/kg, respectively, of purified monoclonal antibody in saline when they were 15 kg. Pigs in groups 10–12 were injected with 0.1, 0.5 or 1.0 mg/kg, respectively, of purified monoclonal antibody in the same volume of saline when they were 60 kg. Group 13 was the control to groups 1–6, and group 14 was the control to groups 7–12. These controls were given the same volume of saline by intraperitoneal or subcutaneous injection. The treatment of the pigs is detailed in **Tables 1** and **2**.

The pigs were slaughtered when they grew to 90 kg and the carcass traits (**Table 3**) were tested according to the method of Xiong and Deng [16].

Table 1 Weight and dosage of antibody given to pigs in treatment groups 1–6 and 13

Group	Pig injection weight (kg)	Dose of injection (mg/kg weight)
1	15	0.1
2	15	0.5
3	15	1.0
4	60	0.1
5	60	0.5
6	60	1.0
13	15	0.0

Table 2 Weight and dosage of antibody given to pigs in groups 7–12 and 14

Group	Pig injection weight (kg)	Dose of injection (mg/kg weight)
7	15	0.1
8	15	0.5
9	15	1.0
10	60	0.1
11	60	0.5
12	60	1.0
14	15	0.0

Table 3 Definitions of abbreviations used to describe the carcass traits of pigs

Abbreviation	Full definition	Unit
LMP	Lean meat percentage	%
FMP	Fat meat percentage	%
BFT	Backfat thickness	cm
LEA	Loin eye area	cm^2
LFW	Leaf fat weight	kg
CFW	Caul fat weight	kg
HW	Heart weight	kg
SW	Spleen weight	kg
KW	Kidney weight	kg
LIW	Liver weight	kg
LUW	Lung weight	kg
DP	Dressing percentage	%

Statistical analysis

Statistical significance of differences between means was assessed with the least square method (GLM procedure, version 4.18; SAS, Cary, USA).

Results

Effects on adipose *in vitro*

During the early stage of culture, stromal-vascular cells differentiated gradually into pre-adipocytes from day 1 to day 5 in serum-containing media. Pre-adipocytes began to accumulate lipid on day 5 of culture and were round or shuttle-like in shape. The monoclonal antibody treatment with complement was carried out on day 5 of culture. On day 8 of culture, in the presence of antibody and complement, pre-adipocyte differentiation gradually stopped and cell disruption and stearolysis appeared [**Fig. 1(A)**]. At the same time the lipid accumulation of these fat cells and the number of well-differentiated fat cells were both significantly reduced ($P<0.01$) compared to the control not treated with antibody or complement [**Fig. 1(D)**]. Treatment with antibody alone did not induce cell lysis [**Fig. 1(B)**], but the number of well-differentiated cells and lipid accumulation was reduced remarkably ($P<0.05$) compared to the control. Treatment with complement alone showed almost the same results as the control [**Fig. 1(C, D)**].

During the next stage of culture (day 8 to day 11), pre-adipocytes gradually differentiated into adipocytes. Cells

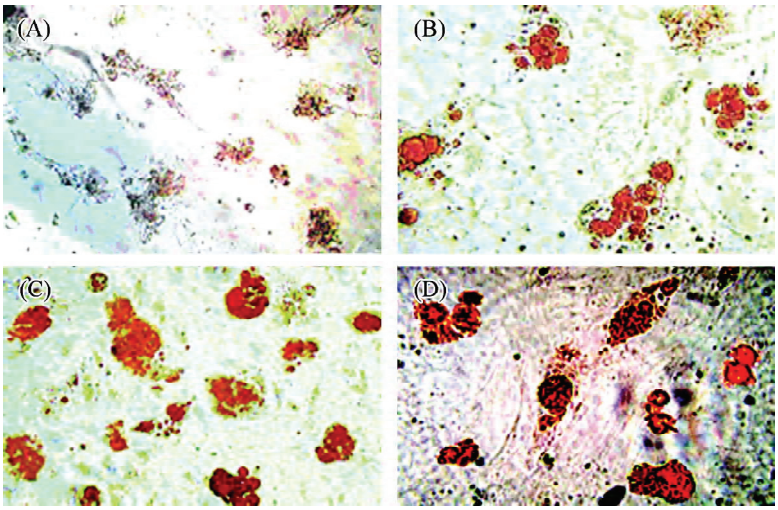

Fig. 1 Micrographs of porcine pre-adipocytes cultured from day 5 to day 8 in serum-supplemented medium and stained for lipids with Oil red O

Cultures were treated with antibody and complement (A), antibody alone (B), or complement alone (C). (D) Control culture without antibody or complement. Magnification, 200×.

appeared round in shape and were filled with numerous lipid droplets in cytoplasm. For the treatment with antibody and complement from day 8 to day 11, a number of adipocytes were disrupted and released an amount of lipid. The well-differentiated cell number was also reduced notably (P<0.01) compared to the control [**Fig. 2(A,D)**]. However, treatment with antibody alone or complement alone did not induce cell disruption and their well-differentiated cell number and lipid accumulation were not significantly changed [**Fig. 2(B,C)**].

During the final days of culture (day 15 to day 20), adipocytes gradually differentiated further into mature adipocytes, the lipid droplets fused and formed large globules, and their nucleus migrated toward the cell periphery. During this stage, mature adipocyte differentiation gradually stopped and most of the cells were fully

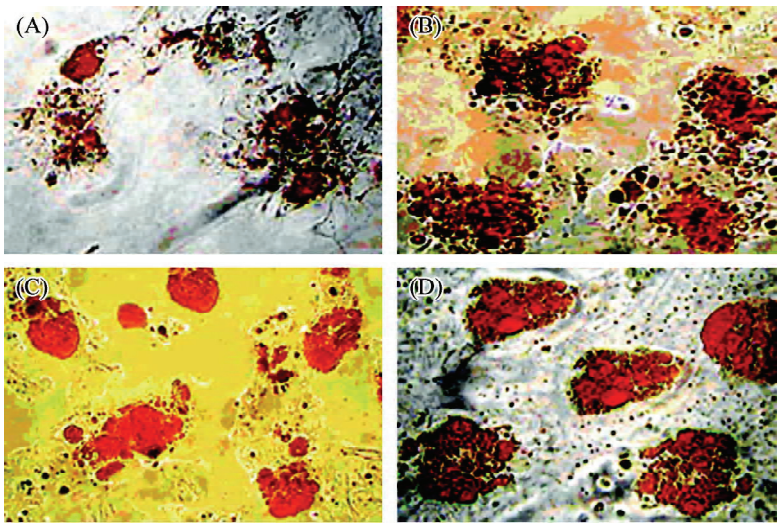

Fig. 2 Micrographs of porcine adipocytes cultured from day 8 to day 11 in serum-supplemented medium and stained for lipids with Oil red O

Cultures were treated with antibody and complement (A), antibody alone (B), or complement alone (C). (D) Control culture without antibody or complement. Magnification, 200×.

filled with multiple lipid droplets. In this stage similar effects were observed as in day 8 to day 11 of culture (**Fig. 3**).

Comparisons of the triglyceride content of adipocytes and numbers of well-differentiated cells for these three treatment cultures are shown in **Figs. 4** and **5**.

Effects on adipose tissues *in vivo*

Micrographs of frozen sections of subcutaneous dorsal adipose tissue indicated that the sizes of adipose tissue cells from pigs injected with 0.5 mg/kg or 1.0 mg/kg of antibody were clearly smaller than that of the control (**Fig. 6**). The size of backfat adipocytes was measured by planimetry after collagenase digestion and cell osmication. Significant differences were found between the control group and treatment groups (**Fig. 7**). The triglyceride content of subcutaneous dorsal adipose tissue was also reduced significantly (P<0.01) by treatment with 0.5 mg/kg and 1.0 mg/kg of antibody (**Fig. 8**).

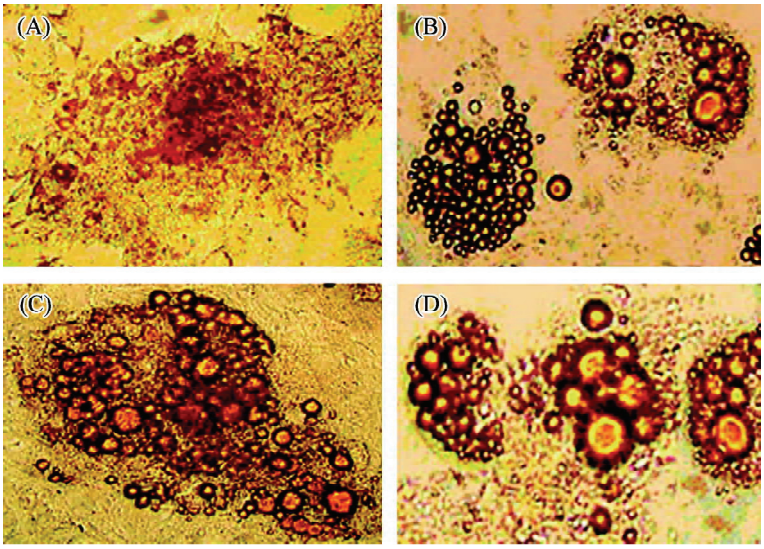

Fig. 3 Micrographs of mature porcine adipocytes cultured from day 15 to day 20 in serum-supplemented medium and stained for lipids with Oil red O

(A) Culture during the last day treated with antibody and complement. (B) Culture during the last day treated with antibody alone. (C) Control culture with complement alone. (D) Control culture without antibody or complement. Magnification, 200×.

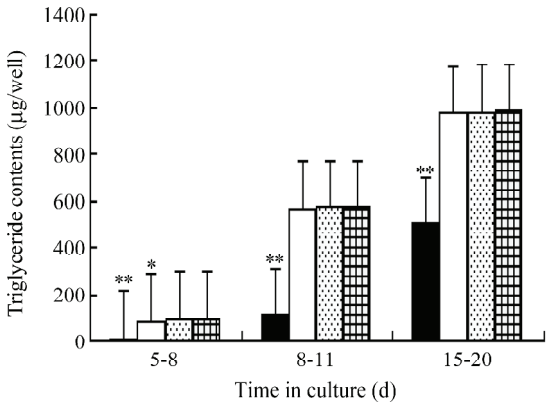

Fig. 4 Triglyceride content of porcine adipocytes for treatment cultures and control

Cells were cultured from day 5 to day 20 in serum-supplemented medium with antibody and complement (■), with antibody alone (□), with complement alone (⁙), or without antibody or complement (▦). Data are presented as the mean±SD of three experiments. *P<0.05 and **P<0.01 versus control group.

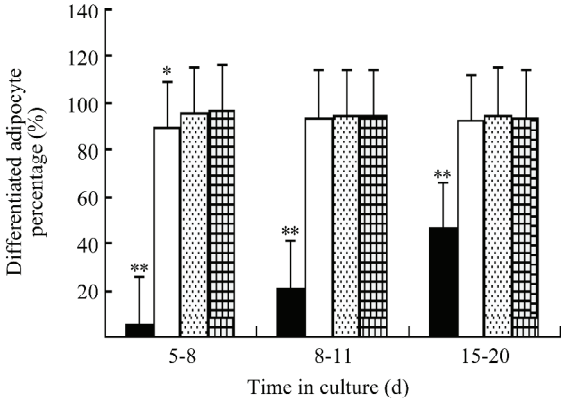

Fig. 5 Comparison of well-differentiated porcine adipocyte numbers in treatment cultures and control

Cells were cultured from day 5 to day 20 in serum-supplemented medium with antibody and complement (■), antibody alone (□), complement alone (⁙), or without antibody or complement (▦) and stained for lipids with Oil red O. Data are presented as the mean±SD of three experiments. *P<0.05 and **P<0.01 versus control group.

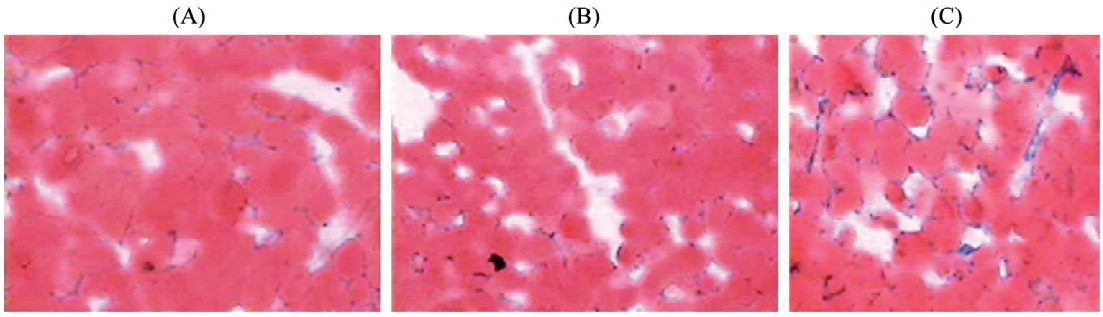

Fig. 6 Micrographs of frozen section of porcine subcutaneous dorsal adipose tissue and stained for lipid using Oil red O

Micrographs show the frozen section from the control pigs (A), pigs treated with 0.5 mg/kg of antibody (B), and pigs treated with 1.0 mg/kg of antibody (C). Magnification, 200×.

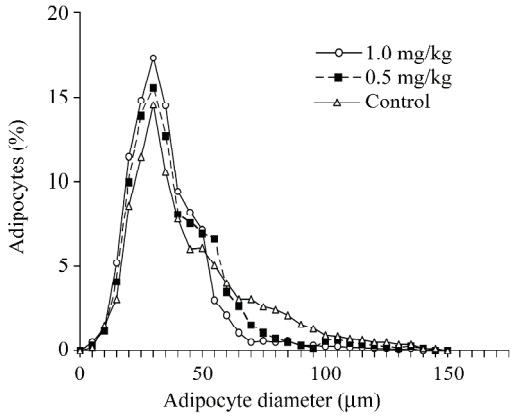

Fig. 7 Comparison of porcine backfat adipocyte sizes in control group and in groups treated with 0.5 or 1.0 mg of antibody per kilogram of body weight (500 cells were measured in each animal)

$P<0.01$ versus control group; $P<0.05$ between treatment groups ($n=6$).

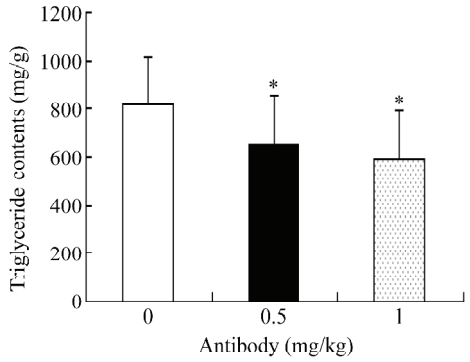

Fig. 8 Comparison of the triglyceride content of subcutaneous dorsal adipose tissue from control pigs and pigs treated with 0.5 or 1.0 mg of antibody per kilogram of body weight (90 kg)

Data are presented as the mean±SD of each group ($n=6$). *$P<0.01$ versus 0 mg/kg antibody group.

Effects of intraperitoneally injected monoclonal antibody on carcass composition

The results showed that this kind of monoclonal antibody against a porcine 40 kDa adipocyte-specific plasma membrane protein could significantly affect some carcass traits of pigs immunized by intraperitoneal injection, especially those animals immunized with 0.5 mg/kg or 1.0 mg/kg of antibody. Compared with the control group, for pigs intraperitoneally immunized with 0.5 mg/kg or 1.0 mg/kg of monoclonal antibody at 15 kg, the lean meat percentage was increased by 8.23% ($P<0.05$) and 9.86% ($P<0.05$), fat meat percentage was decreased by 17.20% ($P<0.05$) and 19.19% ($P<0.05$), backfat thickness was lowered by 16.31% ($P<0.05$) and 19.89% ($P<0.05$), loin eye area was enhanced by 28.08% ($P<0.05$) and 30.52% ($P<0.05$), and leaf fat weight was reduced by 10.89% ($P<0.05$) and 12.84% ($P<0.05$), respectively.

Compared with the control group, for the pigs intraperitoneally immunized with 0.5 mg/kg or 1.0 mg/kg monoclonal antibody at 60 kg, the lean meat percentage was decreased by 6.82% ($P<0.05$) and 7.16% ($P<0.05$), fat meat percentage was lowered by 13.18% ($P<0.05$) and 14.49% ($P<0.05$), backfat thickness was reduced by 16.07% ($P<0.05$) and 18.56% ($P<0.05$), loin eye area was increased by 26.49% ($P<0.05$) and 28.45% ($P<0.05$), and leaf fat weight was reduced by 8.95% and 9.73% ($P<0.05$), respectively. These results are detailed in **Table 4**.

From **Table 4**, it can also be seen that this kind of monoclonal antibody injected intraperitoneally had no significant effect on traits such as heart weight, spleen weight, kidney weight, liver weight, lung weight and dressing percentage. Pigs intraperitoneally immunized with this kind of mouse monoclonal antibody with the dose of 0.1 mg/kg displayed no significant differences to the control group.

Table 4 Effects analyses of mouse monoclonal antibody on carcass composition of pigs injected intraperitoneally

Traits	Group						
	1	2	3	4	5	6	13
LMP (%)	50.05±1.03	52.53±1.43*	53.32±1.13*	49.93±1.34	51.85±1.07*	52.05±1.21*	48.54±1.31
FMP (%)	25.32±0.83	22.47±0.77*	21.51±0.73*	25.58±0.81	23.31±0.84*	22.96±0.86*	26.85±0.79
BFT (cm)	3.36±0.10	2.89±0.15*	2.74±0.13*	3.39±0.09	3.00±0.10*	2.92±0.11*	3.61±0.09
LEA (cm^2)	29.23±1.03	34.62±1.11*	35.28±1.17*	29.15±0.98	34.19±0.93*	34.72±1.02*	27.03±1.14
LFW (kg)	2.53±0.13	2.29±0.11*	2.06±0.11*	2.54±0.11	2.34±0.13*	2.32±0.12*	2.57±0.11
CFW (kg)	2.38±0.11	2.36±0.08	2.33±0.09	2.41±0.09	2.39±0.10	2.36±0.12	2.57±0.10
HW (kg)	275.41±14.97	277.13±14.83	278.17±14.91	274.44±14.92	275.01±14.88	275.48±14.94	274.35±14.91
SW (kg)	121.06±9.27	121.08±9.32	121.04±9.34	121.03±9.30	121.07±9.33	121.05±9.29	121.05±9.30
KW (kg)	266.98±18.32	267.23±18.36	267.35±18.33	267.17±18.35	267.26±18.38	267.33±18.31	267.32±18.33
LIW (kg)	1419.79±112.26	1421.32±112.32	1425.41±112.31	1415.53±112.29	1416.92±112.37	1425.34±112.34	1413.25±112.30
LUW (kg)	1052.52±88.37	1053.16±88.34	1054.39±88.32	1052.43±88.38	1052.92±88.33	1052.62±88.31	1052.83±88.31
DP (%)	72.78±3.13	73.15±3.00	73.68±3.03	72.26±2.83	73.02±3.11	73.14±3.23	71.46±3.07

* $P<0.05$. Data are presented as mean±standard error. Abbreviation definitions are provided in **Table 3**.

Effects of subcutaneously injected monoclonal antibody on carcass composition

Similar results were seen in pigs injected subcutaneously, compared with the control group. For pigs subcutaneously immunized with 0.5 mg/kg or 1.0 mg/kg monoclonal antibody at 30 kg, the lean meat percentage was increased by 6.41% ($P<0.05$) and 7.09% ($P<0.05$), fat meat percentage was decreased by 12.59% and 13.45% ($P<0.05$), backfat thickness was lowered by 20.78% ($P<0.05$) and 24.65% ($P<0.05$), loin eye area was increased by 21.53% ($P<0.05$) and 23.83% ($P<0.05$), and leaf fat weight was decreased by 9.34% ($P<0.05$) and 14.79% ($P<0.05$), respectively. For the pigs subcutaneously immunized with 0.5 mg/kg or 1.0 mg/kg of monoclonal antibody at 60 kg, the lean meat percentage was increased by 5.81% ($P<0.05$) and 6.70% ($P<0.05$), fat meat percentage was decreased by 12.29% ($P<0.05$) and 13.11% ($P<0.05$), backfat thickness was lowered by 16.90% ($P<0.05$) and 19.11% ($P<0.05$), loin eye area was enhanced by 20.90% ($P<0.05$) and 21.61% ($P<0.05$), and leaf fat weight was reduced by 7.39% and 8.56% ($P<0.05$), respectively. These results are detailed in **Table 5**. From **Table 5** it can also be seen that this kind of monoclonal antibody, injected

Table 5 Effects analyses of mouse monoclonal antibody on carcass composition of pigs injected subcutaneously

Traits	Group						
	7	8	9	10	11	12	14
LMP (%)	49.74±1.11	51.65±1.13*	51.98±1.07*	49.57±1.09*	51.36±1.21*	51.79±1.15*	48.56±1.33*
FMP (%)	25.64±0.81	23.47±0.78*	23.24±0.82*	25.72±0.80*	23.55±0.79*	23.33±0.81*	26.84±0.81*
BFT (cm)	3.33±0.11	2.86±0.15*	2.72±0.13*	3.41±0.08*	3.03±0.13*	2.94±0.11*	3.62±0.08*
LEA(cm^2)	28.36±1.12	32.85±1.03*	33.47±1.05*	28.08±0.99*	32.68±1.01*	32.87±1.11*	27.02±1.15*
LFW (kg)	2.56±0.10	2.33±0.14*	2.19±0.13*	2.58±0.11*	2.38±0.09*	2.35±0.12*	2.57±0.12*
CFW (kg)	2.45±0.11	2.42±0.13	2.28±0.10	2.51±0.13	2.46±0.12	2.43±0.11	2.55±0.13
HW (kg)	274.39±14.90	274.91±14.96	275.03±14.86	274.37±14.89	274.82±14.93	274.9±14.94	274.36±14.93
SW (kg)	121.04±9.25	121.09±9.35	121.01±9.34	121.05±9.32	121.06±9.33	121.03±9.34	121.04±9.31
KW (kg)	267.21±18.30	267.27±18.35	267.34±18.28	267.23±18.32	267.29±18.27	267.36±18.31	267.33±18.32
LIW (kg)	1414.56±112.32	1416.73±112.25	1425.28±112.33	1414.41±112.39	1416.25±112.36	1421.71±112.23	1413.24±112.37
LUW (kg)	1052.62±88.29	1052.89±88.37	1053.51±88.33	1053.08±88.26	1053.36±88.36	1053.62±88.35	1052.88±88.23
DP (%)	71.87±3.11	72.43±3.03	72.66±3.07	71.53±3.01	72.25±3.00	72.36±3.09	71.44±3.15

* $P<0.05$. Data are presented as mean±standard error. Abbreviation definitions are provided in **Table 3**.

subcutaneously, had no significant effect on traits such as heart weight, spleen weight, kidney weight, liver weight, lung weight or dressing percentage. Results also indicated that subcutaneous immunization with 0.1 mg/kg of this monoclonal antibody did not significantly affect the carcass traits measured.

Discussion

Our experiment indicated that the complement-mediated cytotoxicity of the monoclonal antibody we prepared previously showed remarkable suppressive effects on the adipocytes *in vitro* and depressive effects on subcutaneous adipose tissue *in vivo*. These were results of adipocyte lysis, reduction of lipid accumulation (mainly about triglycerides), decrease in the number of well-differentiated cells and shrinkage in the size of fat cells. This is in agreement with the research results of Wright and Hausman [8] and de Clercq *et al.* [10], suggesting that we can develop other monoclonal antibodies to suppress pig fat deposition.

Although Wright and Hausman and de Clercq *et al*. [8, 10] pioneered the research on the depressive effects of anti-adipocyte monoclonal antibodies on porcine adipocytes and the development of pig adipose tissue, they only studied their short-term effects. In our experiment, this effect was investigated over a much longer time. Our results suggest that this depressive effect is more significant in the pre-adipocyte stage than in the adipocyte and mature adipocyte stages *in vitro*. *In vivo* our experiment also showed that this monoclonal antibody given to pigs at 15 kg weight had more significant effects on carcass traits than the same dose of antibody given to pigs at 60 kg weight. These results suggest that the earlier treatment of adipocytes with this monoclonal antibody was even more effective than later treatment.

From the results obtained in this experiment it can be seen that treatment with the monoclonal antibody prepared by us could remarkably affect some porcine carcass traits related to fat deposition at 90 kg weight when pigs were immunized with 0.5 mg/kg or 1.0 mg/kg monoclonal antibody. This supported the findings of de Clercq *et al*. [10].

Kestin *et al*. [6] had reported that the effects of subcutaneous injections were limited but intraperitoneal injection was always associated with better and more remarkable effects on carcass traits. Our results in **Tables 4** and **5** also indicate that intraperitoneal injection is superior to subcutaneous injection. Our results also showed that treatment with higher dose monoclonal antibody led to more noticeable effects on carcass traits.

From the results obtained in this experiment it could also be seen that immunization using this monoclonal antibody did not significantly affect any porcine carcass trait other than the traits associated with fat deposition. This concurs with the specificity analysis results of the monoclonal antibody that showed it displayed desirable specificity to plasma membrane proteins of subcutaneous fat cells but not to any protein of cell membrane fractions in other tissue cells [12], suggesting that the antibody produced negligible side-effects to tissues other than fat tissues.

This mouse monoclonal antibody raised against the porcine adipocyte-specific plasma membrane protein showed obvious suppressive effects on porcine adipocytes and fat tissues, with negligible side-effects on other tissues. These results suggest that this monoclonal antibody could be applied to restrain excessive fat deposition in future porcine production.

References

1 Flint DJ, Coggrave H, Futter CE, Gardner MJ, Clarke TJ. Stimulatory and cytotoxic effects of an antiserum to adipocyte plasma membranes on adipose tissue metabolism *in vitro* and *in vivo*. Int J Obes 1986, 10: 69–76

2 Cryer A, Gray BR, Woodhead JS. Study on the characterization of bovine adipocyte precursor cells and their differentiation *in vitro*, using an indirect-labelled-second antibody cellular immunoassay. J Dev Physiol 1984, 6: 159–168

3 Dulor J, Reyne Y, Nougues J. *In vivo* effects of a treatment with antibodies to adipocyte plasma membranes in the rabbit. Reprod Nutr Dev 1990, 30: 49–58

4 Dong J, Froman DP, Hu CY. Development and characterization of polyclonal antibodies against chicken adipocytes. Comp Biochem Physiol A 1991, 99: 195–198

5 Moloney AP, Allen P. Growth and weights of abdominal and carcass fat in sheep immunized against adipose cell membranes. Proc Nutr Soc 1989, 48: 14–21

6 Kestin S, Kennedy R, Tonner E, Kiernan M, Cryer A, Griffin H, Butterwith S *et al*. Decreased fat content and increased lean in pigs treated with antibodies to adipocyte plasma membranes. J Anim Sci 1993, 71: 1486–1495

7 Wright JT, Hausman GJ. Monoclonal antibodies against cell surface antigens expressed during porcine adipocyte differentiation. Int J Obes 1990, 14: 395–409

8 Wright JT, Hausman GJ. Monoclonal antibody-mediated cytotoxicity of adipocytes. Obes Res 1995, 3: 265–272

9 Wu YJ, Wright JT, Young CR, Cartwright AL. Inhibition of chicken adipocyte differentiation by *in vitro* exposure to monoclonal antibodies against embryonic chicken adipocyte plasma membranes. Poultry Sci 2000, 79: 892–900

10 de Clercq L, Mourot J, Genart C, Davidts V, Boone C, Remacle C. An anti-adipocyte monoclonal antibody is cytotoxic to porcine preadipocytes *in vitro* and depresses the development of pig adipose tissue. J Anim Sci 1997, 75: 1791–1797

11 Hao JY, Gao SZ, Wang L, Zhang X, Zhang YJ. Isolation and identification of specific porcine adipocyte membrane protein. Chin J Vet Sci 2004, 24: 571–575

12 Gao SZ, Zhang X, Ge CR, Hao JY, Wang L, Zhang YJ. Preparation and characterization of the specific monoclonal antibodies to porcine adipocyte plasma membrane protein. Chin J Vet Sci 2005, 25: 185–188

13 Hausman GJ, Wright JT, Dean R, Richardson RL. Cellular and molecular aspects of the regulation of adipogenesis. J Anim Sci 1993, 71: 33 –42

14 Ramirez-Zacarias JL, Castro-Munozledo F, Kuri-Harcuch W. Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with Oil red O. Histochemistry 1992, 97: 493–497

15 Seveus L, Johannessen JV. Embedding, sectioning, and staining. In: Johannessen JV ed. Electron Microscopy in Human Medicine. (Vol 1) Instruments and techniques. New York: McGraw-Hill International Book Company 1978

16 Xiong YZ, Deng CY. Principles and Methods of Swine Testing. Beijing: Chinese Agricultural Press 1999

Edited by
Minghua XU