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Nutritional deprivation reduces the transcripts for transcription factors and adipocyte-characteristic proteins in porcine adipocytes

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For an organism to survive during nutritional deprivation, it must be able to regulate the genes involved in energy metabolism. White adipose tissue is an energy source during fasting conditions. In adipose tissue, transcription factors regulate several adipocyte-characteristic proteins involved in differentiation and energy metabolism. We investigated the transcript concentrations of two key transcription factors, as well as the transcript concentrations of several adipocyte-characteristic proteins, and genes involved in adipocyte energy metabolism in the adipose tissue of pigs fasted for 72 hours. Nutritional deprivation resulted in decreased transcript concentrations of the transcription factors, peroxisome proliferator-activated receptor gamma, and CCAAT-enhancer-binding protein alpha. The transcript concentrations of several adipocyte-characteristic proteins, fatty acid synthase, glucose transporter 4, lipoprotein lipase, leptin, and adipocyte fatty acid binding protein were also significantly reduced. The insulin receptor transcript concentration did not change. We conclude that these transcript concentration changes are aimed collectively at adjusting energy partitioning to conserve energy during nutritional deprivation, thereby enabling survival. (J. Nutr. Biochem. 11:139–146, 2000) © *Elsevier Science Inc. 2000. All rights reserved.*

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Introduction

White adipose tissue (WAT) functions to store reserve energy in the form of triglycerides and to mobilize this energy source during nutritional deprivation, allowing for homeostasis and survival. Regulation of the genes involved in energy metabolism in response to fasting or feeding is essential for survival. Several transcription factors form a cascade to regulate each other as well as several adipocyte-characteristic proteins involved in differentiation and energy metabolism. Transcription factors appear to control differentiation by binding to specific response elements in target genes of the differentiating adipocyte.[1] Two nuclear hormone receptor protein families that play important roles in adipocyte differentiation are the peroxisome proliferator-activated receptors (PPAR) and the CCAAT-enhancer-binding proteins (C/EBP). Subfamily members that appear to be involved in adipogenesis are PPARγ, C/EBPβ, and C/EBPα.

Transcription factor C/EBPβ appears early in the adipocyte differentiation process and stimulates the production of PPARγ.[2,3] The transcription factor PPARγ operates as a heterodimer with retinoid X receptor alpha (RXRα), which is activated through binding with a specific ligand. This activated PPARγ-RXRα complex controls the expression of genes containing PPAR response elements.[4] One such adipocyte gene induced early in differentiation is C/EBPα, which appears to sustain the expression of PPARγ. Individually or together, PPARγ and C/EBPα regulate the expression of adipocyte-characteristic genes such as lipoprotein

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lipase (LPL), leptin, adipocyte fatty acid binding protein (aP2), and fatty acid synthase (FAS).[5] The result is adipocyte differentiation, accumulation of triglycerides in these adipocytes, and activation of many of the genes involved in adipocyte metabolism. Ultimately, the result is an increase in total WAT mass.[6]

In response to a fasted state, to enable survival an adaptation mechanism is important for regulating the expression of genes involved in adipocyte energy metabolism. Therefore, we examined the effects of nutritional deprivation on the transcript levels for several of the transcription factors in the adipose tissue of growing pigs. We also examined changes in the transcript levels for several adipocyte-characteristic proteins, as well as transcript levels for genes involved in adipocyte energy metabolism in these same pigs.

Methods and materials

Animals

Twelve crossbred castrated male pigs, aged 8 to 10 weeks and obtained from the Texas Department of Corrections (Huntsville, TX USA), were transported to the animal facility at the Children's Nutrition Research Center. On arrival, the pig weights ranged from 15 to 20 kg. The pigs were given ad libitum access for 7 days to Purina Laboratory Mini-Pig Starter diet #5080 (PMI Feeds, Richmond, IN USA). This diet contains 20.5% crude protein, 4% fat, and 4.1% crude fiber.

Following this 1-week ad libitum access to feed, all pigs were weighed and were individually anesthetized with a cocktail containing ketamine sulfate (16.4 mg/kg) and acepromazine (0.37 mg/kg). Pre-experiment biopsies (−7 days) were removed from each pig in an aseptically prepared region of the dorsolateral neck-shoulder region on the left side. A total of four biopsy samples, each 15 mm in diameter, were taken from each pig. The skin and any muscle were immediately trimmed from each biopsy sample, and the adipose tissue was frozen in liquid nitrogen and stored at −70°C until use. Following the biopsies, each pig received an intramuscular injection containing Flo-Cillin (44,000 U/kg) and Torbugesic (0.1 mg/kg) (Fort Dodge Animal Health, Fort Dodge, IA USA). All pigs were allowed ad libitum access to feed and water for the next 48 hours. At that time, all pigs were fasted overnight for 18 hours, and a pre-experiment fasting blood sample (−4 days) was drawn from the jugular vein of each animal. After the fasting blood sample was obtained, all pigs were again allowed ad libitum access to feed and water for the next 4 days. The 12 pigs were then divided into six pairs of pigs based on similar paired weights (0 day). One of the pigs in each pair was assigned to the fed control group and the other pig, the larger of the pair, was assigned to the fasting group. The fed group ($n = 6$) was housed in the same room, with each pig in an individual cage; all fed control pigs received ad libitum access to feed and water throughout the remainder of the experiment. The fasting group ($n = 6$) was housed in a different room with each pig in an individual cage; these fasting pigs were allowed access to water only. The nutritional deprivation of the fasting group lasted for 72 hours.

At the end of the 72 hours (+3 days), both the fed group and the fasting group were sacrificed with a captive bolt pistol and exsanguinated. Backfat thickness in all pigs was measured 2.5 cm from the midline at the last rib, with the pig placed in a prone position and the front and rear legs facing cephalad. A postexperiment blood sample was collected from each pig at the time of sacrifice. Tissue was removed immediately after sacrifice from the upper and middle subcutaneous adipose tissue of the right side, neck, and shoulder regions. All tissues were trimmed, immediately frozen in liquid nitrogen, and stored at −70°C until use. At the time of the blood sampling, the fed group had ad libitum access to feed and the fasting group had been fasted for 72 hours. The Baylor College of Medicine Animal Care and Use Committee approved the animal use protocols.

Plasma chemistry

Plasma samples were analyzed for glucose, blood urea nitrogen, triglyceride, and total protein using a Vitro 950 Clinical Analyzer (Ortho Clinical Diagnostics, Rochester, NY USA). Nonesterified fatty acids were determined using a Cobas Para II (Roche Diagnostics, Nutley, NJ USA). All automated blood chemistries were performed at Texas Children's Hospital Laboratory (Houston, TX USA). Plasma insulin was manually measured using a radioimmunoassay kit from Linco (#PI-12K, Linco Research, St. Charles, MO USA).

Total RNA extraction from tissue

Total RNA was extracted from the pre-experiment adipose tissue biopsy samples (−7 days) and from the postexperiment adipose tissue samples (+3 days) by the guanidinium-phenol-chloroform extraction method[7] described elsewhere.[8,9] Total RNA was quantified by spectroscopy using the A_{260} for RNA concentration and stored at −70°C until use. The quality of the total RNA was assessed by visual inspection of the 18S and 28S ribosomal bands after denaturing electrophoresis as described elsewhere.[8,9]

Isolation and cloning of probes

The DNA templates for riboprobe transcription were constructed from pig adipose tissue total RNA using reverse transcription and polymerase chain reaction. Each DNA template isolation was performed in the presence of a single pair of gene-specific sense and antisense oligonucleotide primers as previously described.[9,10] Characteristics of the porcine specific probes are presented in *Table 1*. Sequences of the porcine gene fragments for C/EBPα, PPARγ, LPL, 18S ribosomal RNA (18S), and aP2 were previously reported.[10] The PPARγ riboprobe hybridizes to the isoforms gamma 1 and gamma 2. Dr. Scott Mills (Purdue University, West Lafayette, IN USA) provided a partial sequence for the porcine insulin receptor (InsR). Primers were designed from this sequence information and used to isolate the insulin receptor gene fragment indicated in *Table 1*. The FAS gene fragment was obtained from a clone of porcine FAS, which was provided by Dr. Stephen Clarke (University of Texas, Austin, TX USA).[11]

Synthesis of labeled riboprobes and Northern blot analysis

Radiolabeled riboprobes were generated by transcription of the linearized plasmids containing the gene fragment of interest using the Strip-EZ T7 kit (Ambion Inc., Austin, TX USA). Labeled riboprobes generated from this transcription were used for Northern blot analysis. Details of these methods are described elsewhere.[10]

For Northern blot analysis, total RNA (25 μg) from each sample was electrophoresed under denaturing conditions and transferred overnight to Nytran nylon membranes (Schleicher & Schuell, Keene, NH USA) using the Turboblotter Rapid Downward Transfer System (Schleicher & Schuell), as previously described.[10] The membranes were prehybridized in hybridization bottles containing 3.5 mL of Ultrahyb solution (Ambion Inc.) for each 10 × 15 cm membrane used. Prehybridization was carried out

Table 1 Characteristics of porcine-isolated DNA sequences

Porcine probe	Probe size (bp)	Transcript size (kb)	Primer sequences	Source for primers	GenBank accession number	Nucleotide sequence homology
C/EBPα	388	2.7	Sense: 5′-GGTGGACAAGAACAGCAACG-3′ Antisense: 5′-AGGCACCGGAATCTCCTAGT-3′	Human	AF103944	Human = 94% (U34070) Mouse = 92% (M62362)
PPARγ	331	1.9	Sense: 5′-CACAGGCCGAGAAGGAGAAG-3′ Antisense: 5′-ATCTCCTGCACAGCCTCCAC-3′	Human	AF103946	Human = 95% (L40904) Mouse = 92% (U01664)
aP2	296	.7	Sense: 5′-GGCTTTGCTACCAGGAAAGT-3′ Antisense: 5′-GCAGTGACACCATTCATGAC-3′	Bovine	AF102872	Bovine = 90% (X89244) Mouse = 83% (M13385)
LPL	294	3.5	Sense: 5′-GCAGGAAGTCTGACCAATAA-3′ Antisense: 5′-CTTCACCAGCTGGTCCACAT-3′	Human	AF102859	Pig = 98% (X62984) Human = 91% (M15856) Rat = 90% (L03294)
Leptin	277	4.2	Sense: 5′-ATGCGCTCTGGACCCCTGTG-3′ Antisense: 5′-CATTTCTGGAAGGAGACTGGT-3′	Porcine	AF102856	Pig = 100% (U59894) Pig = 77% (U66254) Human = 87% (U18915) Rat = 87% (D49653)
InsR	595	6.2, 8.3	Sense: 5′-TTATCATTGGACCCCTCAT-3′ Antisense: 5′-AACTTCTTGGCGTTCAAGT-3′	Mouse	AF102858	Human = 91% (A18657) Rat = 87% (M29014) Mouse = 89% (J05149)
Glut4	524	2.9	Sense: 5′-CCAACAGATAGGCTCCGAAG-3′ Antisense: 5′-TGGCCAGTTGGTTGAGCGTC-3′	Human	AF141956	Pig = 96% (S71386) Human = 90% (M20747) Rat = 85% (D25861)
18S	649	1.9	Sense: 5′-CTCGATGCTCTTAGCTGAGT-3′ Antisense: 5′-CTAGTTAGCATGCCGAGAGT-3′	Rat	AF102857	Human = 100% (M10098) Rat = 100% (V01270)

C/EBPα–CCAAT/enhancer-binding protein alpha, PPARγ–peroxisome proliferator-activated receptor gamma, aP2–adipocyte fatty acid binding protein, LPL–lipoprotein lipase, InsR–insulin receptor, Glut4 – glucose transporter 4, 18S–18S ribosomal RNA.

at 60°C with continuous rotation for at least 2 hours, at which time the denatured riboprobe (95°C for 10 minutes) was added at a concentration of 2×10^6 cpm/mL of prehybridization solution. This hybridization was carried out overnight at 60°C, at which time the membranes were washed at 60°C for 10 minutes in 2X-SSPE (300 m*M* NaCl, 20 m*M* NaH_2PO_4, 2 m*M* ethylendiamine-tetraacetic acid at pH 7.4). The membranes were then washed twice in 2X-SSPE containing 0.1% sodium dodecyl sulphate for 30 minutes at 65°C. All membranes were sealed in plastic wrap and exposed to a phosphorimaging screen from 20 minutes to 5 days depending on the riboprobe and the relative number of transcript copies expected. Relative abundance of each transcript was then determined using phosphorimagery (Storm 860 with ImageQuant 4.2A software, Molecular Dynamics, Sunnyvale, CA USA). The densitometric value for each individual transcript concentration in a sample lane was normalized by the densitometric value for 18S ribosomal RNA transcript concentration in the same sample lane and presented in arbitrary units. All membranes were hybridized at the same time using the same riboprobe. Following densitometric evaluation with a single riboprobe, the membranes were stripped using Ambion's Strip-EZ RNA kit (Ambion Inc.) per the manufacturer's instructions and reconstituted for reprobing with a different riboprobe. This technique allowed us to reprobe the same membranes several times with different riboprobes. Potentially low copy transcripts were probed first to insure maximal hybridization signals from those transcripts.

Statistical analysis

Statistically significant differences between group means were determined with Student's *t*-tests, using unpaired comparisons. All analyses of data were performed with the Instat program (GraphPad Software, San Diego, CA USA).

Results and discussion

Growth characteristics (Table 2)

The weight range of the pigs ($n = 12$) at the start of the nutritional deprivation (0 day) was 20.95 to 30.75 kg with no significant difference between the mean weights of the pigs destined to be in the fed or fasted groups ($P = 0.37$). The postexperiment fed group (+3 days) continued to gain weight during the 3 days of the experiment, with a mean weight gain of 3.5 ± 1.2 kg (14.2% increase in body

Table 2 Growth characteristics of the pigs

	Control group	Fasted group	Significance
Pre-experimental weight (kg)*	24.7 ± 2.5	26.2 ± 2.8	$P = 0.37$
Post-experimental weight (kg)†	28.2 ± 2.7	21.8 ± 2.8	$P < 0.001$
Post-experimental backfat (mm)‡	8.2 ± 1.2	5.3 ± 1.2	$P < 0.005$

Data represent means and SD from the control group ($n = 6$) and the fasted group ($n = 6$).
*Weights were obtained from the control and fasted groups prior to any nutritional deprivation.
†Weights were obtained at sacrifice from the fasted group after nutritional deprivation for 3 days. Control group (ad libitum access to feed) weights were obtained at sacrifice.
‡Backfat thickness was measured following sacrifice as described in the Methods section.

Table 3 Plasma chemistries

	Pre-experimental groups*			Postexperimental groups†		
	Control	Fasted	Significance	Control	Fasted	Significance
Glucose (mmol/L)	4.0 ± 0.6	3.9 ± 0.5	$P = 0.77$	6.8 ± 0.2	5.5 ± 0.3	$P < 0.01$
Insulin (pmol/L)	15.8 ± 3.1	19.6 ± 4.9	$P = 0.14$	46.9 ± 23.5	19.2 ± 4.6	$P < 0.01$
NEFA (mEq/L)	0.75 ± 0.31	0.82 ± 0.28	$P = 0.71$	0.01 ± 0.0001	0.83 ± 0.09	$P < 0.01$
Triglycerides (mmol/L)	0.69 ± 0.37	0.70 ± 0.18	$P = 0.95$	0.65 ± 0.18	1.10 ± 0.36	$P < 0.05$
Urea nitrogen (mmol/L)	3.7 ± 1.4	3.9 ± 1.2	$P = 0.82$	5.3 ± 0.8	3.9 ± 0.7	$P < 0.05$
Total protein (g/L)	56 ± 2	60 ± 6	$P = 0.23$	60 ± 2	67 ± 5	$P < 0.01$

Data represent means and SD from the control group ($n = 6$) and the fasted group ($n = 6$).
*Fasting samples (18 hr) were collected from the control and fasted groups prior to any nutritional deprivation.
†Fasting samples were collected from the fasted group after 72 hours of nutritional deprivation. Nonfasting samples were collected from the control group (ad libitum access to feed) at the same time.
NEFA–nonesterified fatty acids.

weight). The postexperiment fasted group (+3 days) lost weight after 3 days of nutritional deprivation, with a mean weight loss of 4.5 ± 0.5 kg (16.8% decrease in body weight). The postexperiment final weights (+3 days) between the two groups were statistically different ($P < 0.001$). Backfat thickness (+3 days) was significantly lower in the fasted group compared with the fed group ($P < 0.01$) and represented a 35% decrease in backfat thickness after 3 days of fasting.

Plasma chemistries (Table 3)

There was no significant difference between the pre-experiment (−4 days) baseline fasting blood chemistries of the pigs destined to be in the fed or fasted groups. After the 72-hour fast, plasma glucose, insulin, and blood urea nitrogen were significantly decreased. Nonesterified fatty acids were significantly increased, probably due to increased lipolysis in the fasted group. These results are similar to the blood chemistries obtained after 3 days of fasting in 60 kg and 130 kg pigs.[12] Triglycerides and total protein were significantly greater in the fasted group, but these differences were probably not clinically significant because the values were still in the normal range for pigs. The increase in plasma nonesterified fatty acids and triglycerides in the fasted group compared with the fed group was similar to the results obtained from pigs in a previous study.[13]

Pre-experiment transcript concentrations

There was no significant difference between the pre-experiment biopsy (−7 days) transcript concentrations of the pigs destined to be in the fed or fasted groups (*Table 4*). This suggests that any changes in transcript concentrations following nutritional deprivation were due to the treatment and not due to differences among the pigs prior to dietary changes.

There were no significant differences (*Table 5*) in any transcript concentration measured in adipose tissue from control pigs at day 0 and the same pigs 3 days later. This suggests that any changes seen in the postexperiment fasted group were not due to changes caused by growth of the pigs over the 3 days of the experiment.

Transcription factor transcript concentrations after fasting (Table 6)

The PPARγ transcript concentration was reduced by 37% in the 72-hour fasted pigs compared with the fed pigs. Houseknecht et al.[14] reported a 60% reduction in PPARγ2 transcript concentration and only a 30% reduction in PPARγ1 transcript concentration (not significant) in subcutaneous adipose tissue from 136 kg pigs after fasting for 48 hours. A similar decrease in adipose tissue PPARγ transcript concentration resulted from a 48-hour fast in rats[15] and in mice.[16] In two chronic human studies in which women received very low calorie diets, the PPARγ transcript concentrations were decreased 25% and 13%, respectively.[17,18]

The C/EBPα transcript concentration was decreased 61% after fasting for 72 hours. Expression of C/EBPα is one of the adipocyte genes induced by the activated PPAR-RXR dimer.[5] Therefore, a decrease in PPARγ expression due to nutritional deprivation could result in a decreased expression of C/EBPα. During pig adipose tissue development in

Table 4 Pre-experiment relative transcript concentrations

	Control group	Fasted group	Significance
C/EBPα	506† ± 143	389 ± 144	$P = 0.19$
PPARγ	ND	ND	ND
FAS	213 ± 149	94 ± 50	$P = 0.24$
InsR	401 ± 200	292 ± 88	$P = 0.34$
Glut4	166 ± 70	174 ± 33	$P = 0.83$
LPL	355 ± 96	246 ± 139	$P = 0.15$
Leptin	262 ± 131	198 ± 71	$P = 0.36$
aP2	539 ± 199	348 ± 137	$P = 0.13$

Data represent means and SD from the control group ($n = 6$) and the fasted group ($n = 6$).
*Pre-experiment groups (control and fasted) both had ad libitum access to feed.
†Represents the mean of the densitometric values for the individual sample transcript concentrations normalized to 18S for the same samples and presented in arbitrary units.
C/EBPα–CCAAT-enhancer-binding protein α. PPARγ–peroxisome proliferator-activated receptor γ. ND–not determined. FAS–fatty acid synthase. InsR–insulin receptor. Glut4–glucose transporter 4. LPL–lipoprotein lipase. aP2–adipocyte fatty acid binding protein.

Table 5 Control group relative transcript concentrations

	Pre-experiment	Postexperiment	Significance
C/EBPα	506† ± 143	344 ± 87	$P = 0.08$
PPARγ	ND	ND	ND
FAS	213 ± 149	395 ± 151	$P = 0.19$
InsR	401 ± 200	497 ± 279	$P = 0.56$
Glut4	166 ± 70	186 ± 41	$P = 0.64$
LPL	355 ± 97	316 ± 245	$P = 0.73$
Leptin	262 ± 131	493 ± 389	$P = 0.21$
aP2	539 ± 199	612 ± 187	$P = 0.53$

Data represent means and SD from the control group ($n = 6$) and the fasted group ($n = 6$).
*Postexperiment control group represents the same pigs as the pre-experiment control group but after 72 hours of ad libitum access to feed.
†Represents the mean of the densitometric values for the individual sample transcript concentrations normalized to 18S for the same samples and presented in arbitrary units.
C/EBPα–CCAAT-enhancer-binding protein α. PPARγ–peroxisome proliferator-activated receptor γ. ND–not determined. FAS–fatty acid synthase. InsR–insulin receptor. Glut4–glucose transporter 4. LPL–lipoprotein lipase. aP2–adipocyte fatty acid binding protein.

vivo and differentiation of porcine S/V cells in vitro, the PPARγ transcript concentration was increased before the C/EBPα transcript concentration.[10] This developmental pattern supports the concept of the control of C/EBPα transcripts by PPARγ.

The C/EBPα is not only induced in adipogenesis but also has adipogenic action when expressed at high levels.[19] This requirement for C/EBPα for adipocyte differentiation was demonstrated in experiments in which the expression of antisense C/EBPα RNA in 3T3-L1 preadipocytes prevented differentiation.[20] Disruption of the C/EBPα gene gave rise to mice that failed to develop WAT.[21] The transcription factors C/EBPα and PPARγ appear to act synergistically to trigger adipocyte differentiation. Ectopic expression of either in 3T3-L1 preadipocytes promotes partial differentiation, whereas co-expression promotes the same level of differentiation produced by hormonal inducers.[6,22,23] Two C/EBPα binding sites have been identified in the PPARγ promoter region,[24] allowing for a control mechanism of PPARγ by C/EBPα. A C/EBPα consensus site in the mouse C/EBPα promoter could permit autoregulation by direct binding of C/EBPα.[25] Adipocytes from mice deficient in C/EBPα accumulated fewer lipids and showed less induction of PPARγ, indicating that cross-regulation between C/EBPα and PPARγ is important in maintaining a differentiated state.[26] Our data show significant down-regulation of C/EBPα and PPARγ transcript concentrations as a result of nutritional deprivation, which fits the model of synergism and cross-regulation involving these two transcription factors.

Table 6 Postexperiment relative transcript concentrations

	Control group	Fasted group*	Significance	% Change
PPARγ	593† ± 101	374 ± 129	$P < 0.01$	−37
C/EBPα	344 ± 87	136 ± 62	$P < 0.01$	−61
FAS	395 ± 251	105 ± 54	$P < 0.05$	−73
Glut4	186 ± 41	26 ± 9	$P < 0.01$	−86
LPL	316 ± 245	57 ± 29	$P < 0.05$	−82
Leptin	493 ± 389	98 ± 61	$P < 0.05$	−80
aP2	612 ± 187	232 ± 57	$P < 0.01$	−62
InsR	497 ± 279	596 ± 89	$P = 0.53$	+20

Data represent mean and SD from the control group ($n = 6$) and the fasted group ($n = 6$).
*Fasted group values represent adipose tissue samples following 72 hours of nutritional deprivation and the control group had ad libitum access to feed throughout the experiment.
†Represents the mean of the densitometric values for the individual sample transcript concentrations normalized to 18S for the same samples and presented in arbitrary units.
PPARγ–perixosome proliferator-activated receptor γ. C/EBPα–CCAAT-enhancer-binding protein α. FAS–fatty acid synthase. Glut4–glucose transporter 4. LPL–lipoprotein lipase. aP2–adipocyte fatty acid binding protein.

Adipocyte-characteristic transcript concentrations after fasting (Table 6)

The FAS transcript concentration decreased 73% after fasting. This result is similar to the 75% decrease in FAS transcript concentration seen in mice fasted for 12 hours.[27] In contrast to the well-expressed FAS transcript concentrations in the fed controls, the transcript concentration for FAS was barely detectable in adipose tissue from severely restricted (20% of maintenance) sheep and cattle.[28] The FAS enzyme is a multifunctional protein that may be the rate-limiting step in the production of long-chain, saturated fatty acids and is involved in coordinating fatty acid uptake and storage.[29] The transcription factors PPARγ and/or C/EBPα appear to control the expression of FAS.[29] Decreased FAS transcript concentration in our study probably occurred as a result of the decreased transcript concentrations of PPARγ and C/EBPα in these fasting pigs.

An alternative mechanism for decreased FAS transcript concentration occurs through adipocyte determination and differentiation-dependent factor 1/sterol regulatory element binding protein (ADD1/SREBP1).[27] This transcription factor has been shown to regulate the expression of several genes involved in fatty acid and triglyceride metabolism, including FAS. Expression of ADD1/SREBP1 in adipose tissue of mice was reduced significantly upon fasting and paralleled closely the regulation of FAS. The promoter region of FAS can be transactivated by ADD1/SREBP1. This transcription factor may link changes in nutritional status to the expression of genes that regulate energy metabolism. We did not measure ADD1/SREBP1 transcript concentrations in this study.

The adipose tissue glucose transporter 4 (Glut4) transcript concentration was decreased 86% in fasted pigs compared with fed pigs. The Glut4 transcript concentration was reduced dramatically after 3 days of food deprivation in isolated rat adipocytes.[30] The function of Glut4 is to enhance glucose uptake in response to insulin-stimulated signaling.[31] Its translocation and fusion with the cell membrane facilitate the transport of glucose into the cell. The C/EBPα binds to the promoter region of Glut4,[32] and C/EBPα expression is activated during preadipocyte differentiation just prior to the expression of a Glut4 reporter gene.[32,33] Our data are consistent with these findings,

demonstrating the association of a decrease in C/EBPα transcript concentrations with a concomitant decrease in Glut4 transcript concentration, thereby resulting in a reduced uptake of glucose in the adipocyte during fasting conditions.

The LPL transcript concentrations decreased 82% in fasted pigs compared with control pigs. These findings are similar to the 70% decrease[34] and the 57% decrease[35] in LPL transcript concentrations observed in epididymal fat from rats starved for 72 hours. The LPL activity in the rat studies also decreased 80 to 90% after fasting. The transcript concentration for LPL was barely detectable in adipose tissue from severely restricted (20% of maintenance) sheep and cattle compared with fed controls.[28] LPL is responsible for hydrolysis of triacylglycerols, and changes in local LPL activity can regulate the amount of fatty acids available for storage or oxidation. Regulation of LPL in adipose tissue during fasting is critical for maintaining triglyceride homeostasis. The decrease in LPL activity associated with starvation has been attributed to both pre- and posttranslational mechanisms.[34] The PPARγ and/or C/EBPα transcription factors control the expression of several adipocyte-specific genes, including LPL.[5,29,32] A PPAR response element sequence in the LPL promoter region is capable of conferring PPAR transactivation of the LPL gene in adipocytes.[36] The decrease in LPL transcript concentration seen in our fasted animals was likely due to a decrease in response element induction of LPL because of a decrease in the PPARγ.

The leptin transcript concentration decreased 80% in the fasted group compared with the fed group. Nutritional deprivation for 3 days in 60-kg and 136-kg pigs resulted in a 31% decrease in leptin transcript concentration compared with control pigs.[12] Fasting for up to 72 hours reduced the leptin transcript concentration in adipose tissue of mice by 85 to 90%.[27,37,38] A 47% decrease in leptin transcript concentration was seen in cattle fasted for 48 hours.[39] Women placed on a very low calorie diet for 21 days had 58% less leptin transcript concentration than they did before the diet.[18] In this study, the PPARγ and leptin transcript concentrations were positively associated before ($r = 0.778$, $P < 0.01$) and after ($r = 0.797$, $P < 0.03$) a 21-day very low calorie diet, suggesting that common regulatory mechanisms might control their expression. The proximal promoter region of the leptin gene was found to contain a functional C/EBPα-binding site that mediates transactivation of the leptin gene by C/EBPα.[40] In our study, a decrease in PPARγ and C/EBPα transcript concentration could explain the decrease in leptin transcript concentration during fasting conditions.

In the fasting group, the aP2 transcript concentration decreased 62% compared with the control group. Adipocyte fatty acid binding proteins bind long-chain fatty acids as they enter the cell and direct the fatty acids to the appropriate cytosolic or membrane-bound protein for metabolic activation.[41] The fatty acid binding proteins in adipose tissue contain functional PPAR response element sequences in the promoter regions of their genes.[42] A C/EBPα consensus site has also been found in the promoter region of the aP2 gene that links the expression of C/EBPα with the expression of aP2 in mouse preadipocytes.[43] The decrease we observed in aP2 transcript concentration probably results from down-regulation of PPARγ and/or C/EBPα transcripts.

The porcine adipocyte tissue insulin receptor transcript concentration did not change in the fasted group compared with the fed group. In rats, mothers fed a low-protein diet (8%) during pregnancy and lactation had a threefold increase in insulin receptors compared with control (20% protein) subjects.[44] Rats starved for 72 hours had a twofold increase in insulin receptor number in epididymal fat.[45] These studies measured insulin receptor number, whereas we measured the steady-state insulin receptor transcript concentration. If the insulin receptor transcript concentration represents the protein level, there was no increase in insulin receptor number in fasted porcine adipocytes.

Nutritional deprivation for 72 hours resulted in mobilization of energy reserves from the adipose tissue of these fasted pigs, as indicated by a 35% loss of backfat thickness. Nutritional deprivation resulted in significant decreases in the transcript levels of two key transcription factors active in the control of adipocyte differentiation process, namely PPARγ and C/EBPα. In addition, there was a significant decrease in the transcript levels of genes involved in adipocyte energy metabolism (FAS, Glut 4, LPL, leptin, and aP2). These changes appear to involve adaptations that are aimed at adjusting energy partitioning to conserve energy in the face of nutrient deprivation, therefore allowing for survival.

Chronic restriction of feed intake in pigs to 50% of ad libitum for 5 weeks did not significantly change the concentration of transcripts for C/EBPα, PPARγ, LPL, or InsR.[46] The transcript concentrations for Glut4, FAS, and leptin tended to be slightly reduced in the restrictively fed compared with the ad libitum control pigs. Only the aP2 transcript concentration was significantly reduced ($P < 0.01$) with chronic feed restriction. These data suggest that the transcript concentrations are maintained in a homeostatic condition during long-term dietary restriction, whereas nutritional deprivation for 3 days significantly altered the concentration of the same transcripts.

The present study presents the concomitant measurement of the transcript levels of key transcription factors as well as the transcript levels of several metabolic enzymes during nutritional deprivation. The time course resulting in the marked decrease in transcript levels observed in this experiment was not studied, but could provide important information concerning the sequence in decrease of the measured transcripts and their resulting relationships.

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