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Mice Deficient in Phosphofructokinase-M Have Greatly Decreased Fat Stores

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Abstract

Synthesis of triacylglycerol requires the glucose-derived glycerol component, and glucose uptake has been viewed as the rate-limiting step in glucose metabolism in adipocytes. Furthermore, adipose tissue contains all three isoforms of the glycolytic enzyme phosphofructokinase (PFK). We here report that mice deficient in the muscle isoform PFK-M have greatly reduced fat stores. Mice with disrupted activity of the PFK-M distal promoter were obtained from Lexicon Pharmaceuticals, developed from OmniBank OST#56064. Intra-abdominal fat was measured by magnetic resonance imaging of the methylene proton signal. Lipogenesis from labeled glucose was measured in isolated adipocytes. Lipolysis (glycerol and free fatty acid release) was measured in perifused adipocytes. Intra-abdominal fat in PFK-M–deficient female mice (5–10 months old) was 17 ± 3% of that of wild-type littermates ($n = 4$; $P < 0.02$). Epididymal fat weight in 15 animals (7–9.5 months) was 34 ± 4% of control littermate ($P < 0.002$), with 10–30% lower body weight. Basal and insulin-stimulated lipogenesis in PFK-M–deficient epididymal adipocytes was 40% of the rates in cells from heterozygous littermates ($n = 3$; $P < 0.05$). The rate of isoproterenol-stimulated lipolysis in wild-type adipocytes declined ~10% after 1 h and 50% after 2 h; in PFK-M–deficient cells it declined much more rapidly, 50% in 1 h and 90% in 2 h, and lipolytic oscillations appeared to be damped ($n = 4$). These results indicate an important role for PFK-M in adipose metabolism. This may be related to the ability of this isoform to generate glycolytic oscillations, because such oscillations may enhance the production of the triacylglycerol precursor **α**-glycerophosphate.

Introduction

Synthesis of triacylglycerol in adipocytes requires metabolism of glucose to α-glycerophosphate (α-GP) to provide the glycerol component. The rate-limiting step of glucose metabolism in the adipocyte is considered to be glucose transport (1), and the regulation of the adipocyte- (and muscle-) specific transporter Glut4 by insulin is a major part of the control of

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triacylglycerol synthesis by the hormone. A role of the key glycolytic enzyme phosphofructokinase (PFK) in the regulation of adipocyte metabolism has not been well established.

There are three mammalian isoforms of PFK with differing tissue distribution (2,3). Adult muscle has only the M-type subunit, and liver mainly the L-type subunit. Brain and most other tissues, including fat, have C-type as well as M- and L-type subunits, which associate into homo- and heterotetramers. (The term "C-type" has generally been used in rodents; in human, this isozyme has been referred to as "P," for platelet, or "F," for fibroblast.) The three isoforms are transcribed from separate genes and have slightly different apparent molecular weights. The PFK-M–deficient mice studied here have a disrupting tag inserted near the distal promoter that interferes with transcription from that site (4). As a result, tissues that rely on the distal promoter have greatly reduced PFK-M; for example, there is 99% reduction of PFK-M in brain, but only ~50% reduction in total PFK activity, because of the presence of the other isoforms. On the other hand, muscle and heart, which use the muscle-specific proximal promoter in addition, have less dramatic reduction in PFK-M, if any.

PFK isozymes have the same set of activators and inhibitors; however, there are some large differences in affinity for certain regulators. In particular, skeletal muscle PFK is strongly activated by micromolar levels of its product fructose-1,6-bisphosphate (5), a property that can lead to oscillatory behavior of glucose metabolism (6,7). Glucose-dependent oscillations in lipolysis have been observed in perifused isolated adipocytes, suggesting underlying oscillations in glucose metabolism (8,9), and these may in part explain lipolytic oscillations also seen *in vivo* (10). Interestingly, the PFK-M–deficient mice are shown to have considerably reduced fat stores, and their isolated adipocytes exhibit decreased lipogenesis with and without insulin, with a normal degree of stimulation. Lipolysis and lipolytic oscillations are damped in the PFK-M–deficient mouse adipocytes. PFK-M and oscillatory behavior may therefore be of importance in adipocyte metabolism.

Methods and Procedures

Animals

Heterozygous mice with a disrupting tag inserted in the noncoding region of the *PFK-M* gene (OmniBank OST#56064, 50% C57Bl/6 albino, 50% 129svEvBrd) were initially received from Lexicon Pharmaceuticals (The Woodlands, TX). OmniBank is a library of >200,000 mouse embryonic stem cell clones, each containing a gene trap insertion (created by insertional mutagenesis) in a single gene (11). OST#56064 was the only match to the *PFK-M* gene. The mice were bred in-house at Boston University Medical Center to generate wild-type and homozygous PFK-M–deficient mice; in some cases heterozygous littermates were used as controls if wild-type littermates were not available. (Note: "PFK-M" has previously been used to refer to the homotetramer M_4 or muscle PFK, especially in kinetic studies. In this article, "PFK-M deficient" technically refers to loss of the subunit isoform, as detected by western analysis; this implies not only the loss of M_4 but also of M-containing heterotetramers in tissues expressing other isoforms.) Mice used were bred from heterozygotes to avoid any effects of maternal hyperglycemia, because mild hyperglycemia was noted in homozygous females (4). Mice were genotyped with the nptII Invader Assay from Third Wave Technologies (Madison, WI). A couple littermate pairs of younger age (3–4.5 months) were examined, illustrating some diminution of epididymal fat with little difference in body weight; however, most measurements were done on mice aged 7–10 months, where differences were more striking. All procedures were approved by the Boston University Medical Center Animal Care and Use Committee.

Magnetic resonance imaging

Images and spectra were obtained using a Bruker 11.7 (500 MHz for proton) wide bore magnetic resonance spectrometer, with imaging accessories. All experiments were performed in a MicroMouse probe with 30-mm diameter sample chamber. Images for abdominal fat were obtained using the RARE sequence with repetition time 2,500 ms and echo time 12 ms (for "effective echo time" of 48 ms). Matrix size was 256 with a field of view of 2.5 cm. Twenty-four slices were obtained with a slice thickness of 1 mm, running from the top of the diaphragm down roughly to the top of the leg muscles. Spectra were obtained using the PRESS sequence. Repetition time was 1,000 ms and echo time was 15 ms. Triacylglycerol content of liver and muscle was also determined by taking proton spectra of excised tissue and comparing the size of the methylene peak with that of a triacylglycerol standard (olive oil) placed next to the sample. For the organs, 2-mm cubic voxels were used. For the olive oil external standard, 0.5-mm voxels were obtained, 512 averages for each.

Epididymal fat

Epididymal fat pads were excised by cutting right above the epididymis. Both pads were taken and weighed together, as there was no obvious difference between them.

Adipocyte perifusion studies

Adipocytes were isolated and perifused and evaluated as described previously (8,9). In brief, adipocytes were isolated from mouse epididymal fat pads, employing collagenase digestion. After washing, 400 μl of packed cells were loaded onto a column 0.7 cm in diameter and 4 cm high. The perifusing buffer consisted of Krebs–Ringer bicarbonate buffer (final pH 7.4 at 37 °C) composed of the following: 120 mmol/l NaCl, 25 mmol/l $NaHCO_3$, 5 mmol/l KCl, 1.2 mmol/l KH_2PO_4, 1.2 mmol/l $MgSO_4$, 2.5 mmol/l $CaCl_2$, 20 mmol/l 3-(*N*-morpholino) propanesulfonic acid, and 1.0% bovine serum albumin that had been purified by charcoal treatment to remove lipids and then dialyzed against Krebs–Ringer bicarbonate buffer. The cells were perifused at a flow rate of 0.7 ml/min for 30 min to allow for equilibration; then samples were taken for 60 min to determine basal lipolysis. The cells were then perifused with isoproterenol for 2 h to measure stimulated lipolysis. Samples of the effluent were collected every minute and assayed for free fatty acids and glycerol. Pulse analyses of the oscillations in fatty acid and glycerol release were performed using ULTRA (pulse detection algorithm obtained from E. Van Cauter, Department of Medicine, University of Chicago, Chicago, IL) (12).

Cell size

Adipocytes were isolated and plated on 96-well plates for examination under a Nikon inverted microscope eclipse TE200 that is equipped with a camera and SPOT RT software for automatically calculating the diameter of an indicated cell. The samples chosen for this analysis were from a litter where two PFK-deficient mice each had about one-fourth of the epididymal fat weight of the wild type (a bigger difference than the average), so if the difference was largely due to a difference in cell size, this should have been detectable, as a difference in diameter by a factor of the cube root of ¼, or ~0.63.

Measurement of lipogenesis

Incorporation of radioactivity from [^{14}C]glucose into total lipid was determined by incubating adipocytes in Krebs–Ringer bicarbonate buffer containing [^{14}C]glucose (2 mmol/l,100 μCi/μmol), with or without insulin, for 2 h. The incubation was terminated by addition of organic-based scintillation fluid, MicroScint E (Perkin Elmer, Boston, MA). The samples were vortexed and left for 60 min at room temperature to allow the aqueous and organic phases to separate.

The radioactivity in the upper phase was then determined by scintillation counting in a β counter. For cell number, an aliquot of the adipocytes was measured on a hemocytometer.

Food consumption and basal metabolic rate

Food consumption was measured over 6 days. A powdered version of the standard chow was placed in small food consumption jars (Unifab no. 148-WM; Unifab, Kalamazoo, MI) suspended in the cages of the individually housed animals. Food amount was weighed every other day and replenished. Basal metabolic rate was measured over 4 h using a computer-integrated Oxymax Open Circuit Calorimeter System (Columbus Instruments, Columbus, OH) linked to collection chambers to quantify VO_2, VCO_2, respiratory exchange ratio, and heat production.

Statistics

Values are reported as mean ± s.e.m. Comparisons were made using Student's *t*-test.

Results

Decreased fat stores in PFK-M–deficient mice

The magnetic resonance images in Figure 1 are representative cross-sectional slices through female littermates. Bright (hyperintense) areas represent fat because of the intensity of the methylene proton signal there. The internal areas of white are the intra-abdominal fat, whereas the outer band of white is the subcutaneous fat, sandwiched between the skin and the underlying muscle layer. (Different depots of intra-abdominal fat, such as omental, mesenteric, and perirenal, could not be adequately separated by magnetic resonance imaging in mice.) By setting a suitable threshold value for fat and integrating the areas in 24 sequential slices, the fat volume was calculated (Table 1). The intra-abdominal fat volume in the PFK-M–deficient mice was 17 ± 3% of wild type ($n = 4$; $P < 0.02$), including one pair with only 10% difference in body weight. Subcutaneous fat was also greatly reduced.

Triacylglycerol content of liver and muscle was quantified by obtaining proton spectra of excised tissue and comparing the intensity of the methylene peak with that of a vial of olive oil placed next to it. The contents per volume of liver and muscle from PFK-M–deficient mouse Q11 were, respectively, a half and one-third of the values for the wild-type littermate.

The deficiency was also seen in the weights of excised epididymal fat (Figure 2), and even when there was little difference in body weight. In a 4.5-month and a 3-month PFK-M–deficient mouse, epididymal fat was two-thirds that of wild-type littermate; in one case the deficient mouse weighed 5% less and in the other case 3% more than the wild type. (This agrees with our original observations on this colony that there was no consistent difference in body weight between PFK-M–deficient and wild-type mice up to 6 months of age (4).) Three 7-month PFK-M–deficient mice weighed 10% less and had half the epididymal fat of the wild-type littermate. Greater differences were seen in some of the older animals. On average, the 17 PFK-M–deficient mice in Figure 2 had 38 ± 4% of the epididymal fat of the wild-type or heterozygous littermate ($P < 0.002$). The large reduction of PFK-M, but not PFK-C or PFK-L, in epididymal fat in these mice is illustrated in Figure 3.

The decreased epididymal fat weight appears to be a result of decreased adipocyte size rather than of decreased cell number. Measurement of the diameters of 90 isolated adipocytes from a wild-type mouse (second 7-month litter in Figure 2) gave an average diameter of 28 ± 0.4 (s.e.) μm, vs. 17 ± 0.3 μm for 122 cells in a preparation from the two PFK-M–deficient littermates. If the cells are considered spherical, then the volume of the PFK-M–deficient cells should be $(17/28)^3$ or ~0.22 times that of the wild-type cells. This corresponds fairly well with

the lower epididymal fat weight of the two PFK-M–deficient mice, which averaged 0.24 times that of the wild-type mouse.

Decreased lipogenesis in isolated adipocytes from PFK-M–deficient mice

Basal and insulin-stimulated lipogenesis rates in isolated epididymal adipocytes from PFK-M–deficient mice were 40–50% of the corresponding rates in adipocytes from heterozygous littermates (Table 2). (There were no wild-type littermates.) There was a similar fold stimulation by high insulin, indicating that an insulin sensitive step (perhaps glucose transport) is still limiting in the PFK-M–deficient cells. The PFK-M–deficient adipocytes were perhaps stimulated more, certainly not less, at the intermediate concentration of insulin than the control cells.

Altered lipolysis in perifused adipocytes from PFK-M–deficient mice

As shown in Figure 4, when wild-type adipocytes were stimulated with isoproterenol, glycerol release as well as fatty acid release was fairly sustained, declining ~10% after 1 h and 50% after 2 h. In contrast, the release of both glycerol and fatty acids dropped off rapidly in PFK-M–deficient adipocytes, declining 50% in 1 h and 90% in 2 h. Similar results were seen in three other experiments, and average values are shown in the right panel. The decrease in lipolysis is not due to depletion of fat stores, which at the end of the experiment amounted to 6.9 ± 0.4 and 7.1 ± 0.5 μmol/0.1 ml packed cells (n = 3) for PFK-M–deficient and wild-type cells, respectively, ~10 times the amount released during the experiment. Lipolytic oscillations also appear to be damped in the PFK-M–deficient adipocytes (Table 3).

Food consumption and basal metabolic rate

The decreased fat stores in the PFK-M–deficient mice could be due to decreased food consumption or increased basal metabolic rate. We therefore compared these parameters in three PFK-M–deficient and three heterozygous female mice (wild-type littermates not being available), two of each from one litter and one of each from another litter. Although there was a great difference in abdominal fat and a lesser, not quite significant difference in average body weight, there was no obvious difference in food consumption or metabolic rate when calculated on a per-animal basis (Table 4). When calculated on a per-weight basis, food consumption and metabolic rate were both somewhat higher in PFK-M–deficient mice, but the difference did not reach statistical significance. In these animals, the liver triacylglycerol content in the PFK-M–deficient animals was one-tenth that of the heterozygotes.

Discussion

These PFK-M–deficient mice are the result of a disrupting tag inserted near the distal promoter, leaving the proximal, muscle-specific promoter functional (4). The PFK-M–deficient mice are remarkable in having considerably reduced fat stores, as shown by the magnetic resonance imaging of intra-abdominal fat (reduced ~80%) and the weight of excised epididymal fat (reduced ~65%). This was surprising, because although glucose metabolism provides the α-GP needed for triacylglycerol synthesis, the rate-limiting step for glucose metabolism in the adipocyte is considered to be glucose transport, largely under the control of the insulin-sensitive glucose transporter Glut4 (1). Lipogenesis in isolated epididymal adipocytes from PFK-M–deficient mice was 40% of normal, correlating with the similarly reduced weight of the epididymal fat; yet the fold stimulation by insulin was normal, indicating conserved regulation by an insulin sensitive step. Furthermore, fat has the other two subunit isoforms of PFK, PFK-C and PFK-L (2;Figure 3). So one is left with the consideration that there is something special about PFK-M. A major kinetic difference among the isoforms is that PFK-M is strongly activated by its product fructose-1,6-bisphosphate, a property that can lead to the generation of oscillations in glycolysis, as shown in muscle extracts (6,7). Such oscillations involve high

peaks in the glycolytic intermediates, including dihydroxyacetone phosphate, and NADH (nicotinamide adenine dinucleotide, reduced form). The high peaks in dihydroxyacetone phosphate and NADH, which are the substrates for α-GP dehydrogenase, could lead to enhanced production of α-GP. Indeed, a large step in α-GP was seen during the time overlap of the dihydroxyacetone phosphate and NADH peaks (6). In a muscle extract experiment where glucose 1,6-bisphosphate was added to prevent feedback activation of PFK by fructose- 1,6-bisphosphate, the oscillatory glycolysis was converted to steady-state behavior and the high peaks in dihydroxyacetone phosphate were eliminated, whereas average glycolytic flux as indicated by lactate accumulation was unchanged (7,13); however, the accumulation of α-GP was decreased at least 50% (V. Andres, V. Schultz, and K. Tornheim, unpublished data). Thus, in the context of the adipocyte, impaired glycolytic oscillations could lead to a decreased production of α-GP and hence triacylglycerol at the same glycolytic rate under the control of Glut4.

In previous papers (8,9), it was shown that perifused isolated rat adipocytes exhibited oscillations in lipolysis. The dependence of the lipolytic oscillations on the presence of glucose, as well as observed oscillations in lactate output, suggested the involvement of the glycolytic oscillator. The occurrence of oscillatory glucose metabolism in adipocytes was also indicated by the early observations of CO_2 production by Lipkin *et al*. (14). The model proposed to explain the lipolytic oscillations (8,9) was that glycolytic oscillations, which involve oscillations in dihydroxyacetone phosphate and NADH, would produce oscillations in the level of α-GP. Pulses of α-GP in turn would lead to pulsed removal of long chain acyl CoA (LC-CoA) into glycerides. As LC-CoA is an inhibitor of some lipases, such as hormone-sensitive lipase (15), oscillations in the levels of LC-CoA should lead to oscillations in lipase activity and hence in the release of glycerol and fatty acids by the adipocyte. The data presented here show similar lipolytic oscillations in mouse adipocytes, and that the oscillations were damped in the PFK-M–deficient cells. Most striking was the large reduction in lipolysis that developed in the PFK-M–deficient adipocytes during the isoproterenol stimulation. A likely explanation for this decreased lipolysis is that a decreased α-GP production due to impaired glycolytic oscillations leads to a progressive accumulation of inhibitory levels of LC-CoA. The decrease in lipolysis was not due to depletion of fat stores, which were 10 times the amount released during the experiment. These data thus support an important role for PFK-M and oscillations in adipocyte metabolism.

Viewed at the level of the whole animal, another simple possible explanation for the decreased fat stores would be a decrease in food consumption or increased metabolic rate in the PFK-M–deficient mice. However, this seems some-what counterintuitive. If there were a defect in glucose signaling in the brain indicating fuel lack, one might expect an increase rather than a decrease in food consumption. Again, brain has the other two subunit isoforms, which are actually increased in the PFK-M–deficient mice (4). The available data did not show an obvious change in food consumption or basal metabolic rate on a per-animal basis (Table 4), though small changes could have been missed. Normalization on a per-weight basis did give an increase in metabolic rate in the PFK-M–deficient animals that did not reach statistical significance; however, there was a similar increase in food consumption, and it is not clear whether such normalization would be appropriate in this fat-deficient model. In any case, the measurements of lipogenesis and lipolysis in isolated adipocytes demonstrate changed metabolism at the level of the fat cell. It is perhaps also worth noting that certain other major tissues are likely to have normal metabolism in these PFK-M–deficient mice: muscle and heart, because of preserved transcription of PFK-M from the proximal promoter (4), and liver, because of the dominance of the L-isoform in that tissue. In contrast, in PFK-M–deficient humans, muscle glycolysis is blocked, and such subjects have usually been identified because of difficulties in exercising.

PFK-M–deficient mice were previously shown to have elevated blood glucose and impaired glucose tolerance (4). Basal insulin levels were, if anything, higher not lower. The fold rise in insulin in the glucose tolerance test was lower, though the absolute levels were not significantly lower. Glucose-stimulated insulin release in isolated islets was also diminished; islet PFK-M was greatly decreased, but the levels of the other two isoforms were increased, as in brain. Because lipolysis and fatty acids can potentiate insulin release (16), it is quite possible that the insulin secretory defect in these mice is secondary to alterations in lipid metabolism, rather than the other way around. Again, the metabolic differences seen in the isolated adipocytes argue that there is an alteration at the level of the fat cell and that PFK-M is important in adipocyte metabolism.

Acknowledgments

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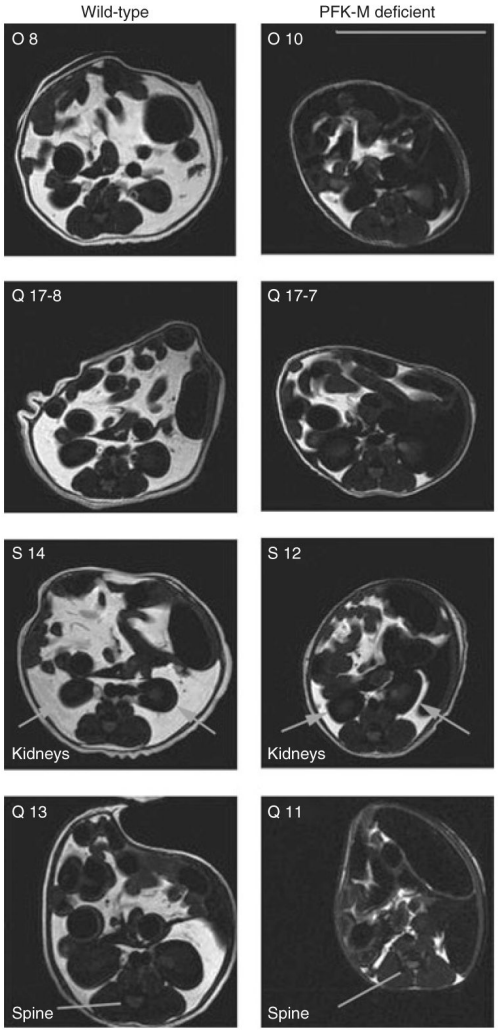

Figure 1.
Methylene proton magnetic resonance images of intra-abdominal fat in female PFK-M–deficient and wild-type littermates. The T1-weighted images shown represent 1-mm cross-sectional slices at the level of the left renal pelvis. Bright (hyperintense) areas represent fat because of the intensity of the methylene proton signal there. Calculated volumes of intra-abdominal fat and subcutaneous fat (the outer band of white) integrated over 24 such slices are given in Table 1, together with the ages and body weights of the mice. Mouse identification numbers are shown in white in the upper left corner of each image. The bar in the upper right panel is 2 cm; all images are on the same scale. PFK, phosphofructokinase.

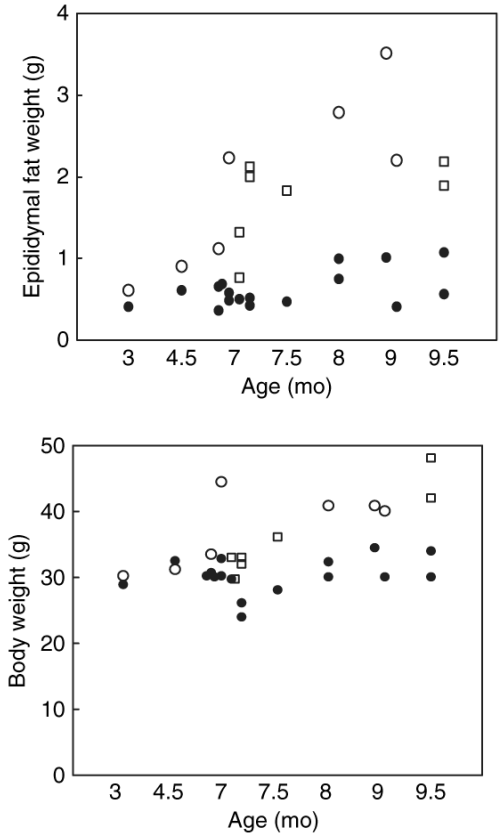

Figure 2.

Epididymal fat and body weights of wild-type (open circles), heterozygous (squares), and PFK-M–deficient (filled circles) littermates. Littermates are grouped in vertical columns, at the indicated age of killing. Heterozygotes were used as controls if there were no wild type in the litter. The 15 PFK-M–deficient mice aged 7–9.5 months had 34 ± 4% of the epididymal fat of their wild-type or heterozygous littermates; the average for all 17 PFK-M–deficient mice shown was 38 ± 4% of the littermate controls ($P < 0.002$). PFK, phosphofructokinase.

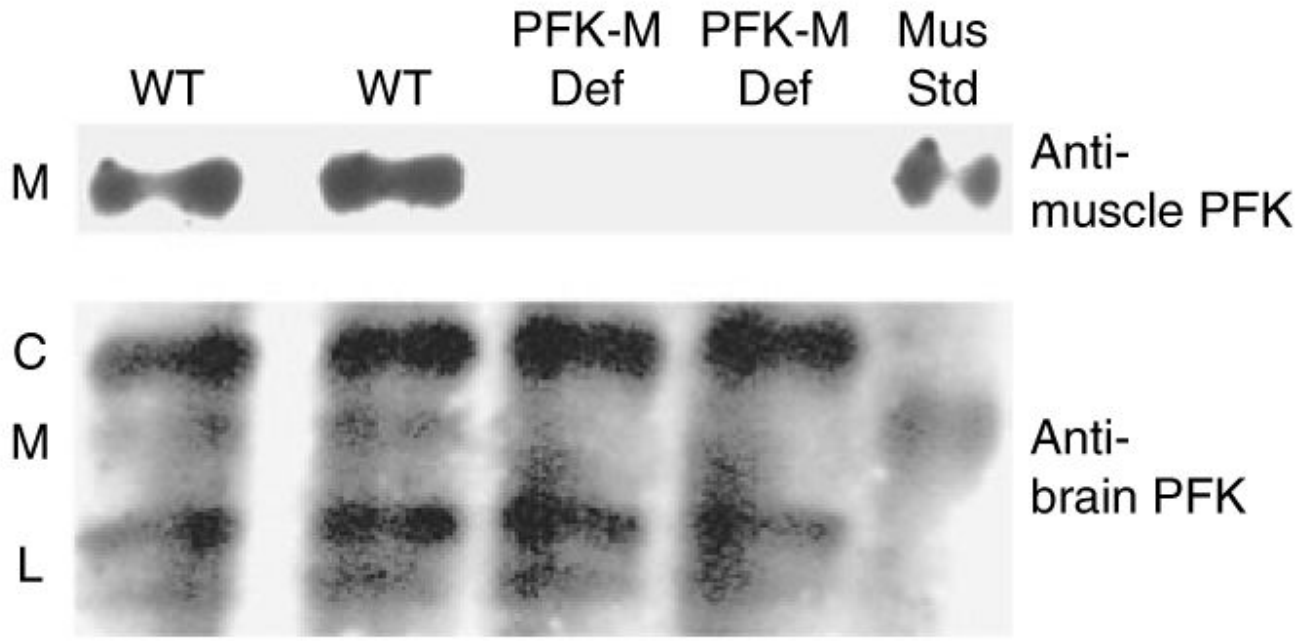

Figure 3.
Western blot of phosphofructokinase (PFK) in epididymal fat (90 μg protein) from wild-type (WT) and PFK-M–deficient (Def) mice. The lane on the right is a skeletal muscle standard (wild type, 1 μg protein). The upper panel shows the blot probed with a PFK-M–specific antibody (hence M-type subunit only), whereas the lower panel shows the blot probed with an anti-brain PFK antibody that reacts with all three PFK subunit isoforms, C, M and L, as indicated on the left (4).

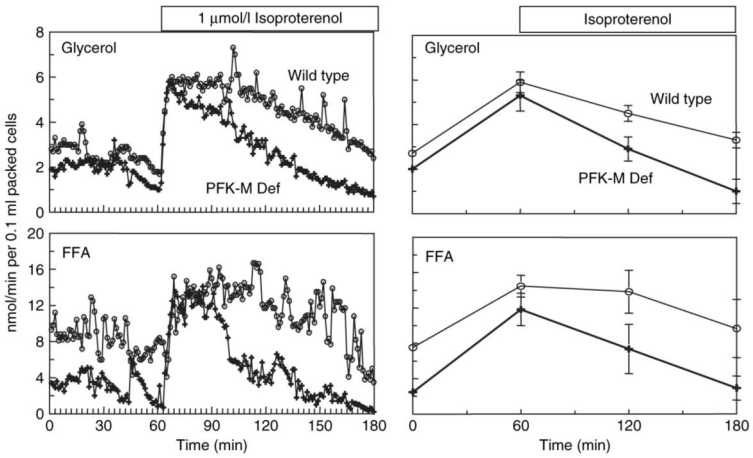

Figure 4.

Lipolysis from perifused adipocytes, stimulated with isoproterenol in the time interval indicated. A single experiment is shown in the left panel, and mean data from three to four experiments are shown in the right panel. Calculated amplitudes and frequencies of oscillations are shown in Table 3. Def, deficient; FFA, free fatty acids.

Table 1

Intra-abdominal and subcutaneous fat in wild-type (WT) and PFK-M–deficient (Def) littermates

Mouse no.	Genotype	Age (months)	Body weight (g)	Intra-abdominal fat (cc)	% of WT	Subcutaneous fat (cc)	% of WT
O8	WT	10	30	3.32		1.23	
O10	Def	10	24	0.39	12	0.17	14
Q17-8	WT	7	26	3.89		3.08	
Q17-7	Def	7	24	0.85	22	0.82	27
S14	WT	5	30	2.72		0.99	
S12	Def	5	22	0.63	23	0.42	42
Q13	WT	8.5	26	1.17		0.6	
Q12	WT	8.5	26	1.9		0.6	
Q11	Def	8.5	18	0.17	11	–	–

Fat volumes were calculated from the magnetic resonance imaging data illustrated in Figure 1 for these mice, using 24 sequential slices. Subcutaneous fat was calculated by subtracting the intra-abdominal fat from the total. For mouse Q11, the subcutaneous fat was too small to be accurately determined. Percentage of WT = 100 (Def fat volume)/(WT littermate fat volume). Average Def intra-abdominal fat was 17 ± 3% of that of WT ($n = 4$; $P < 0.02$).

PFK, phosphofructokinase.

Table 2

Lipogenesis in heterozygous and PFK-M–deficient adipocytes

	Heterozygous	PFK-M deficient	% of Het
Basal	1.9 ± 0.4	0.8 ± 0.1[a]	42
100 μU/ml insulin	2.3 ± 0.2	1.2 ± 0.1[a]	52
10 mU/ml insulin	3.5 ± 0.1	1.3 ± 0.1[a]	38

Data are average ± s.e. (n = 3), calculated as pmol glyceride/cell/2 h, for isolated adipocytes from littermates 7–9.5 months. % of Het = 100 (PFK-M deficient)/(heterozygous).

PFK, phosphofructokinase.

[a] Significantly different from heterozygous, $P < 0.05$.

Table 3

Basal and stimulated (1 μmol/l isoproterenol) lipolytic oscillations in perifused wild-type (WT) and PFK-M–deficient (Def) adipocytes

	Concentration (nmol/min)		Amplitude (nmol/min)		Pulse duration (min)		Pulse frequency (pulses/h)	
	WT	**Def**	**WT**	**Def**	**WT**	**Def**	**WT**	**Def**
Glycerol								
Basal	2.5 ± 0.2	2.0 ± 0.2[a]	0.39 ± 0.11	0.35 ± 0.16	5.5 ± 0.1	5.5 ± 0.1	8 ± 1	8 ± 1
Stimulated	4.6 ± 0.2[b]	3.0 ± 0.2[a,b]	0.54 ± 0.11[b]	0.44 ± 0.09[b]	5.5 ± 0.1	5.5 ± 0.2	10 ± 1	9 ± 1
Fatty acids								
Basal	8.9 ± 0.4	3.1 ± 0.2[a]	1.62 ± 0.08	0.84 ± 0.21[a]	5.2 ± 0.2	5.4 ± 0.1	10 ± 1	10 ± 1
Stimulated	15.9 ± 2.1[b]	9.7 ± 2.6[a,b]	2.79 ± 0.29[b]	1.54 ± 0.21[a,b]	5.0 ± 0.1	5.2 ± 0.1	11 ± 1	10 ± 1

Data reported as average ± s.e. (n = 4) for 0.1 ml packed cells, calculated from the experiments illustrated in Figure 4.

[a] Significantly different from wild type.

[b] Significantly different from basal.

Table 4

Food intake and basal metabolic rate in female littermates (12.5–14 months; $n = 3$)

	Heterozygous	PFK-M deficient	*P* value
Abdominal fat (cc)	3.5 ± 0.2	1.5 ± 0.5	<0.02
Body weight (g)	27.7 ± 0.6	21.5 ± 2.6	0.08
Food intake (g/day)	4.7 ± 0.4	4.4 ± 0.8	0.79
Per 30 g body weight	5.1 ± 0.4	6.1 ± 0.7	0.25
Basal metabolic rate (kcal/h)	0.42 ± 0.06	0.40 ± 0.05	0.82
Per 30 g body weight	0.46 ± 0.08	0.57 ± 0.05	0.31

PFK, phosphofructokinase.