Lysosomal Dysfunction Results in Altered Energy Balance

Josh C. Woloszynek, Trey Coleman, Clay F. Semenkovich, and Mark S. Sands

From the Departments of Medicine, Genetics, and Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Missouri 63110

The mucopolysaccharidosis (MPS) type VII mouse was originally described as the adipose storage deficiency mouse because of its extreme lean phenotype of unknown etiology. Here, we show that adipose storage deficiency and lower leptin levels are common to five different lysosomal storage diseases (LSDs): MPSI, MPSIIIB, MPSVII, Niemann-Pick type A/B, and infantile neuronal ceroid lipofuscinosis. Elevated circulating pro-inflammatory proteins (VCAM1 and MCP1) were found in multiple LSDs. Multiple anti-inflammatory strategies (dexamethasone, MCP1 deficiency, M3 expression) failed to alter adiposity in LSD animals. All of the models had normal or greater caloric intake and lower to normal metabolic rate, fasting plasma glucose, non-esterified fatty acids, cholesterol, and triglycerides. Triglycerides were lower in the livers of MPSI mice, and the trend was lower in the muscle. Lipid absorption and processing in MPSI mice were indistinguishable from those in normal mice following oral gavage of olive oil. The increased lean mass of MPSI and MPSIIIB mice suggests a shift in adipose triglycerides to lysosomal storage. In agreement, MPSI livers had a similar total caloric content but reduced caloric density, indicating a shift in energy from lipids to proteins/carbohydrates (lysosomal storage). Enzyme replacement therapy normalized the caloric density within 48 h without reducing total caloric content. This was due to an increase in lipids. Recycling of stored material is likely reduced or nonexistent. Therefore, to maintain homeostasis, energy is likely diverted to synthesis at the expense of typical energy storage depots. Thus, these diseases will serve as important tools in studying the role of lysosomal function in metabolism and obesity.

EXPERIMENTAL PROCEDURES

Animal Models, Husbandry, and Common Treatments—The MPSI, MPSIIIB, MPSVII, NPAB, and INCL colonies are maintained as pedigreed colonies through strict brother-sister matings at Washington University by M. S. S. Affected and control animals have similar plasma triglycerides and significantly higher non-esterified fatty acids compared with normal mice (7). Thus, it appears that these mice are mobilizing their adipose stores. However, the cause or purpose of the mobilization is not understood. Changes in adiposity may not be surprising in LAL mice because the primary defect is in an enzyme responsible for normal lipid metabolism. However, our current understanding of the metabolic effects of lysosomal storage, especially on lipid metabolism, is limited.

We report here that lysosomal storage results in a deficiency in adipose deposition regardless of the material being stored. MPSI, MPSIIIB, MPSVII, Niemann-Pick type A/B (NPAB), and infantile neuronal ceroid lipofuscinosis (INCL) mice have significantly reduced adiposity and were analyzed with a variety of metabolic tests. The results suggest that lysosomal storage is an energy storage depot that is inaccessible to the cell and body but requires energy to maintain, thereby contributing negatively to energy balance. As this negative contribution grows, it is likely to affect animal viability. Finally, this finding suggests that higher nutrient-rich caloric intake might impact progression of LSDs.
mice were identified by PCR genotyping, with the exception of the INCL colony. INCL mice are bred as homozygotes, and a separate C57BL/6 colony served as a source of controls. All mice were fed high fat (9%) PicoLab Mouse Diet 20 (product code 5058) ad libitum (LabDiet, PMI Nutrition International, St. Louis, MO) from weaning. Unless stated otherwise, MPSI, MPSVII, NPAB, and INCL mice were analyzed at 5 months of age (~23 weeks), and MPSIIIB mice were analyzed at 7 months of age (~30 weeks). For fasting experiments, food was withheld from the animals for at least 4 h.

**Plasma Chemistries**—Depending on the experiment and the mouse model analyzed, whole blood was harvested in EDTA either by heart puncture or from the saphenous vein of fasted animals. Blood obtained from mice bled from the saphenous vein was collected in heparinized capillary tubes (Fisher). Blood obtained by heart puncture was collected through a 26-gauge needle attached to a 1-ml syringe. Plasma was collected by centrifugation at 2940 × g for 5 min. Total cholesterol (Sigma), triglycerides (Sigma), glucose (Sigma), and non-esterified fatty acids (Wako Chemicals USA Inc., Richmond, VA) were analyzed using commercial kits according to manufacturers’ specifications.

**Dual Energy X-ray Absorptiometry Scanning**—The body composition of anesthetized animals was determined by dual energy x-ray absorptiometry (GE Lunar Corp., Madison, WI). Dual energy x-ray absorptiometry analysis is a noninvasive technique that allows for differentiation between lean tissue and fat tissue (10). Adiposity is calculated as the percentage of the body that is fat.

**Food Intake**—Animals were housed individually. For a period of either 5 (MPSI) or 7 (MPSIIIB, MPSVII, NPAB, and INCL) consecutive days, weighed food was provided, and what was left was exchanged and reweighed 23–25 h later. Food consumed was normalized to body weight on the day of collection. The data reported represent the food intake on the final day, which allowed time for the animals to acclimate to the isolation and daily manipulation.

**Indirect Calorimetry**—A single-chamber indirect calorimetry system (Columbus Instruments, Columbus, OH) was used to analyze the metabolic rate in mice in a fed state after a short acclimation period. The volume of oxygen consumed per body weight (ml/g/h) is reported. The last five readings (every 30 s) were averaged.

**Tissue Triglycerides**—Flash-frozen liver and muscle tissue from MPSI and control mice in a fed state were homogenized in a 2-ml mixture of CHCl3/MeOH (2:1, v/v). Samples were incubated overnight at 4 °C. Subsequently, water was added, and samples were vortexed vigorously. After high speed centrifugation, the organic layer was extracted (500–800 µl) and transferred to a fresh tube. One microliter of organic solution containing triglycerides was analyzed in triplicate as described above under “Plasma Chemistries.”

**Bomb Calorimetry**—Whole livers (including gall bladders) from fasted 23-week-old MPSI and control animals were freeze-dried. The whole sample was then ignited in a sealed oxygen-filled metal bomb submerged in a water bath of known volume (NP Analytical Laboratories, St. Louis). The whole system was maintained at a constant temperature. The heat generated by combustion was calculated from the rise in temperature of the water bath, taking into account the heat of ignition. One cal is defined as the amount of energy needed to raise the temperature of 1 g of water by 1 °C.

**Glucose and Insulin Tolerance Tests**—Glucose tolerance testing was performed on fasted MPSI and control animals at 24–27 weeks of age, and insulin tolerance testing was performed at 25–28 weeks of age. Glucose tolerance testing was performed first, followed by insulin tolerance testing at least 1 week later. These tests were performed essentially as described previously (11).

**Lipid Absorption Test**—Fasted mice were injected intravenously with Triton WR-1339 (12.5 mg/0.2 ml of saline; Tyloxapol, Sigma) to block lipoprotein lipase-dependent lipolysis. Without delay, a blood sample was collected from the saphenous vein into heparinized microhematocrit tubes. Blood was expelled into 0.2-ml tubes and spun immediately at 2490 × g for 5 min to obtain plasma. Subsequently, 100 µl of olive oil was administered by oral gavage. Additional blood samples were collected hourly for a total of 4 h from the saphenous vein and processed as described above. Plasma was flash-frozen in liquid N2. This experiment was performed essentially as described previously (12).

**“Western Diet” Treatment**—Twelve-week-old MPSI and control mice were fed a Western diet (TD88137, Harlan Teklad, Madison, WI), in which 42% of the calories come from fat, for 11 weeks.

**Multianalyte Profile Testing**—Fasted animals were bled either from the saphenous vein (live animals) or by heart puncture (CO2-asphyxiated terminal animals), and plasma was obtained and processed as described above under “Plasma Chemistries.” Frozen plasma was submitted to Rules-Based Medicine, Inc. (Austin, TX), for multianalyte profile analysis.

**Enzyme Replacement Therapy**—Recombinant human α-L-iduronidase (Aldurazyme) expressed in and purified from Chinese hamster ovary cells was a kind gift of BioMarin Pharmaceutical Inc. (Novato, CA). Four MPSI mice received a single injection of 10,000 units of α-L-iduronidase diluted in 300 µl of dilution buffer (150 mM NaCl, 10 mM Tris (pH 7.5), and 1 mM β-glycerophosphate) via their tail veins. Six control and four MPSI mice received sham injections of 300 µl of dilution buffer via their tail veins. All mice were killed 48 h post-injection by CO2 asphyxiation.

**Biochemistry**—Portions of spleen from six sham-treated control mice, four sham-treated MPSI mice, or all four enzyme replacement-treated MPSI mice were flash-frozen in liquid nitrogen and stored at −80 °C. The samples were homogenized in buffer containing 150 mM NaCl, 10 mM Tris (pH 7.5), 1 mM dithiothreitol, and 0.2% Triton X-100 and assayed for the amount of total protein using the Coomassie dye binding assay (Bio-Rad). α-L-Iduronidase activity was determined using the fluorescent substrate 4-methylumbelliferyl α-L-iduronide. The results are reported as specific activity (nanomoles of 4-methylumbelliferyl released per h/mg of total protein).

**Histopathology**—Portions of spleen were individually immersed in 2% glutaraldehyde and 4% paraformaldehyde in phosphate-buffered saline and embedded in Spurr’s resin. Sec-
tions of tissue (0.5 μm thick) were stained with toluidine blue and evaluated for lysosomal storage by light microscopy.

Statistical Analysis—All data points presented are the means ± S.D. Statistical significance (p < 0.05) was determined using Student's two-tailed t test assuming equal variance.

RESULTS

Dual energy x-ray absorptiometry demonstrated that all five mouse models of LSD analyzed had significantly (p < 0.05) decreased adiposity compared with normal control animals (Fig. 1A). Each model also had significantly (p < 0.05) less fat mass (data not shown). There were no morphological differences in adipocytes from MPSI mice and age- and sex-matched normal littermates upon examination by light microscopy (data not shown). Changes in lean mass varied between animal models. Two models (MPSVII and INCL) had significantly reduced lean mass; two models (MPSI and MPSIIIIB) had significantly increased lean mass; and NPAB mice were not different from normal mice (data not shown). The reduced adiposity correlated with a significant (p < 0.05) decrease in circulating leptin levels in all models tested (Fig. 1B). MPSI and MPSIIIIB mice had normal body weight, and MPSVII, NPAB, and INCL mice were significantly smaller than the normal control mice (Fig. 1C). Therefore, lysosomal storage and/or lysosomal dysfunction results in widespread failure of adipose tissue deposition regardless of the primary enzyme defect.

Reduced adiposity due to dysfunctional or absent adipocytes can result in severe insulin resistance (13). MPSI mice had normal fasting plasma insulin levels (Fig. 2A). Furthermore, MPSI mice had normal responses to both glucose (Fig. 2B) and insulin tolerance tests (Fig. 2C). Therefore, it appears that MPSI mice suffer from depletion and/or a failure of triglyceride deposition in adipocytes and not dysfunctional adipocytes. Reduced adiposity could be due to reduced caloric intake or lipid malabsorption. Four of the models had significantly (p < 0.05) increased food intake, and MPSIIIB mice had normal food intake (Fig. 3). When challenged with olive oil gavage in the context of lipoprotein lipase inhibition with Triton WR-1339, MPSI and control animals had the same absorption and processing rate of triglycerides (Fig. 4A). If there is increased demand for energy elsewhere in the body causing depletion or if there is malabsorption of nutrients other than triglycerides, then providing a larger caloric intake via lipids should allow for triglyceride deposition in adipocytes. When fed a Western diet (42% calories from fat) for 11 weeks, MPSI and control mice had significantly (p < 0.05) higher adiposity compared with genetically matched mice fed standard chow (9% calories from fat) (Fig. 4B). Thus, the adipose deficiency appears to be caused by adipocyte-extrinsic factors.

We screened the blood for ~60 different circulating proteins that might impact adiposity. The levels of tumor necrosis factor-α, interferon-γ, and interleukin-6 were either unchanged or undetectable in all five models (supplemental Table 1). VCAM1 and MCP1 levels were increased in four of the five models (supplemental Fig. 8). MCP1 has known effects on adipocytes (14, 15). However, MCP1 deficiency in MPSI or NPAB mice failed to increase their adiposity (supplemental Fig. 9). Other chemokines were elevated in MPSI and MPSIIIIB mice (supplemental Table 1) and might modulate adipocyte function. Therefore, we utilized chemokine-binding protein M3 transgenic mice to inhibit a wide variety of chemokines in MPSI and control mice. Chronic expression of M3 had no effect on MPSI adiposity.

FIGURE 1. LSD causes decreased adiposity and leptin levels. A, adiposity was determined by dual energy x-ray absorptiometry. Data are reported as a percentage of the normal ± S.D. Normal is defined as the mean value of control animals. All models were significantly (p < 0.05) different from control animals (CON. A) mice (n = 5–29). B, leptin levels determined by multianalyte profile testing. Data are reported as a percentage of the normal ± S.D. Normal is defined as the mean value of control animals. All models were significantly (p < 0.05) different from control (CON) mice (n = 5–29). C, body weights on the final day of food intake analysis were measured (n = 5–26). *p < 0.05.
Finally, chronic treatment with the steroid dexamethasone also failed to increase adiposity in MPSI mice (data not shown).

Higher diversion or mobilization of lipids, normally destined for adipocyte deposition, could be due to a higher metabolic rate, storage of triglycerides elsewhere, and/or storage of material in a way that is inaccessible to the body. Closed circuit indirect calorimetry was used to measure the metabolic rate in all five models. MPSI, MPSVII, and INCL mice had a signifi-

*FIGURE 2. MPSI mice have a normal response to insulin challenge. A, insulin levels in MPSI and normal control mice were not significantly different as determined by multianalyte profile testing (n = 5 each). uIU, micro-international units. B and C, neither glucose tolerance testing nor insulin tolerance testing, respectively, was significantly different between MPSI (●; n = 8) and normal control (■; n = 13) mice.

*FIGURE 3. LSD results in normal or increased food intake. In all five models of LSD, daily food consumption (grams/day) was measured and normalized to body weight. Data are reported as a percentage of the normal ± S.D. (n = 4–26). Normal is defined as the mean value of control animals. *, p < 0.05.

*FIGURE 4. MPSI mice have normal lipid absorption and increased fat deposition after high fat challenge. A, there was no significant difference in circulating triglyceride levels between MPSI (filled bars) and normal control (stippled bars) animals (n = 3 each) following oral gavage of olive oil. B, there was a significant increase in adiposity observed in both MPSI and control animals fed a Western diet compared with animals fed a 9% fat diet. Data are reported as a percentage of the normal ± S.D. Normal is defined as the mean value of control animals fed a 9% fat diet (n = 6). Filled bar, 9% fat diet-fed MPSI mice (n = 5); checkered bar, 42% fat diet-fed MPSI mice (n = 6); hatched bar, 42% fat diet-fed control mice (n = 5). *, p < 0.05.
LSD Causes Leanness

This study has demonstrated that profound adipose deficiency is a component of LSD. Previous studies of MPSVII and LAL mice had described a severe reduction in adipose tissue of unknown etiology (5, 7–9). In the case of MPSVII mice, it was commonly believed that the adipose storage deficiency phenotype was due to a reduction in caloric intake caused by illness. Determining the cause of the adipose deficiency associated with significantly (p < 0.05) reduced metabolic rate, whereas MPSIIIB and NPAB mice were indistinguishable from control animals (Fig. 5). All models had normal or reduced levels of plasma triglycerides, non-esterified fatty acids, glucose, and cholesterol (Table 1). In addition, triglyceride levels were significantly lower in the liver, and the trend was lower in the muscle of MPSI mice (Fig. 6). Therefore, if energy is being stored elsewhere in the body, it must be in a form other than triglycerides.

Organomegaly is a common feature of LSD (16). Livers from MPSI mice weighed significantly (p < 0.05) more than control livers, yet had similar percent water (Table 2). Total liver caloric content, as determined by bomb calorimetry, was similar in both groups. However, MPSI livers had a lower caloric density (Table 2). This finding is indicative of a shift from a higher caloric-dense material (i.e. lipid) to a lower caloric-dense material (i.e. protein/carbohydrate), which explains some of the hepateomegaly. Thus, some of the energy that could be in the form of lipids is still present within the animal as protein/carbohydrate. These data are consistent with the nature of the primary storage material in MPSI mice (glycosaminoglycans, a complex polysaccharide). Thus, lysosomal storage would be an energy storage depot that is inaccessible to the cells and body. Enzyme replacement therapy should allow affected cells to access this depot. Forty-eight h after intravenous enzyme replacement therapy, the spleens of MPSI mice contained 150% normal enzyme levels and had a significant reduction in histologically demonstrable storage. It has been shown previously that the liver takes up more recombinant enzyme than the spleen and is nearly cleared of lysosomal storage by 48 h following a single injection of β-glucuronidase into the MPSVII mouse (17). After 48 h, the hepateomegaly (excess mass) was reduced by 56% (p < 0.05) (Table 2) in the MPSI mice that received enzyme. However, the liver dry weight and total caloric content were not reduced, and the percent water remained similar to those in normal control mice. Following treatment, the caloric density (kilocalories/g) returned to normal (p < 0.993). These data are consistent with a reduction of glycosaminoglycan storage and an accumulation of lipid.

**DISCUSSION**

This study has demonstrated that profound adipose deficiency is a component of LSD. Previous studies of MPSVII and LAL mice had described a severe reduction in adipose tissue of unknown etiology (5, 7–9). In the case of MPSVII mice, it was commonly believed that the adipose storage deficiency phenotype was due to a reduction in caloric intake caused by illness. Determining the cause of the adipose deficiency associated with

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**TABLE 1**

| Plasma chemistries | Glycerides | NEFAs | Glucose | Cholesterol |
|--------------------|-----------|-------|---------|-------------|
| MPSI 76.4 ± 13.0° | 81.3 ± 19.4 | 72.0 ± 17.0° | 73.0 ± 7.3° |
| MPSIIIB 95.7 ± 17.6 | 103.2 ± 23.3 | 95.3 ± 7.4 | 100.3 ± 14.6 |
| MPSVII 39.8 ± 5.2° | 68.5 ± 10.2° | 81.3 ± 7.7° | 41.4 ± 8.4° |
| NPAB 65.5 ± 146° | 84.5 ± 163° | 87.3 ± 15.3 | 91.6 ± 20.8 |
| INCL 83.9 ± 22.3 | 100.0 ± 11.0 | 108.9 ± 7.3 | 100.4 ± 6.8 |

*Values indicate a significant difference (p < 0.05) from normal control animals.

**TABLE 2**

| Liver caloric values | Wet weight | Dry weight | kcal/liver | kcal/g | Protein/carbohydrate | Fat |
|----------------------|------------|------------|------------|-------|----------------------|-----|
| MPSI (n = 8)          | 1.736 ± 0.129° | 0.450 ± 0.085° | 2.28 ± 0.48 | 5.06 ± 0.14° | 62.5° | 37.5° |
| Control (n = 10–11)   | 1.572 ± 0.139 | 0.384 ± 0.055 | 2.08 ± 0.28 | 5.39 ± 0.21 | 53.9 | 46.1 |
| MPSI with 48-h ERT (n = 4) | 1.533 ± 0.009° | 0.474 ± 0.007° | 2.55 ± 0.03° | 5.39 ± 0.09° | 53.7 | 46.3 |

*Percent protein/carbohydrate and percent fat were derived from kilocalories/g using 4 kcal/g of protein/carbohydrate and 9 kcal/g of fat according to the following equation: 4X + 9(1 – X) = kcal/g.

†Values indicate a significant difference (p < 0.05) from normal control animals.

‡Values indicate a significant difference (p < 0.05) from untreated MPSI animals.
LAL deficiency is complicated by the very fact that triglycerides and cholesterol esters are the primary storage material. In this study, we have shown that lysosomal storage and/or lysosomal dysfunction results in widespread failure of adipose tissue deposition regardless of the primary enzyme defect.

The adipose deficit is not the result of reduced caloric intake or increased metabolic rate (all models) or lipid malabsorption (in the case of MPSI mice). In addition, the insulin sensitivity of the MPSI mice suggests normal adipocyte function. This conclusion is substantiated by the increased adiposity of MPSI mice upon Western diet challenge. Our data do not discriminate decreased lipid deposition in adipocytes from increased mobilization. Although MCP1 has been shown to inhibit adipocyte differentiation in vitro (14, 15), elevated pro-inflammatory proteins do not appear to play a major role in modulating the abnormal adiposity observed in this study. Circulating non-esterified fatty acid was normal or lower in all animal models, suggesting that the adipose deficiency is not the result of increased mobilization. However, it is possible that an increase in tissue utilization is keeping pace with or outpacing increased mobilization. Induced expression of lipoprotein lipase as measured by microarray analysis in the livers of MPSI, MPSIIIB, and MPSVII mice is suggestive of an increased tissue demand for lipids (data not shown). In the case of MPSI mice, the possible increased tissue demand for lipids did not correlate with an increase in triglyceride levels in those tissues. Both MPSI and MPSIIIB mice had normal body weights, suggesting a shift in mass from fat to other tissues. Furthermore, MPSIIIB mice had normal metabolic parameters except for an increase in lean tissue mass. With regard to mass balance, this would suggest that some of the energy normally found as fat stores is still present in the animals in a form other than triglycerides. This idea is supported by the observation of a shift in energy from lipids to proteins/carbohydrates in the livers of MPSI mice. The normalization of the liver caloric density 48 h after enzyme replacement in MPSI mice suggests that the increased proteins/carbohydrates are the stored glycosaminoglycans, a complex polysaccharide. Therefore, lysosomal storage is an energy storage depot that is inaccessible to the cells and body.

A major function of the lysosome is to recycle macromolecules presumably to reduce the demand for the energy intensive de novo synthesis of raw materials. Furthermore, more of the energy budget of the cell is being diverted to both the management of lysosomal storage (increased protein and membrane synthesis) and the production of the raw material no longer available through recycling (Fig. 7). Enzyme replacement therapy allows affected cells to access this energy depot and reduces the metabolic shifts in energy use. As the glycosaminoglycans are catabolized to monosaccharides and transported to the cytosol, the cell will no longer need to devote as much energy to their de novo synthesis, especially during the initial surge. We hypothesize that this will allow traditional cellular energy stores to accumulate to normal levels. Our enzyme replacement experiment supports this view. For example, 48 h after a single treatment, the total liver caloric content was similar to that in untreated mice; however, the energy density (kilocalories/g) was higher and indistinguishable from that in control mice. Thus, there is a higher level of lipid present in the liver. The rapid increase in the lipid fraction could be due to conversion of storage material (glycosaminoglycans and/or membrane) to lipids, reduced catabolism of lipids, and/or an increased influx of lipid. Thus, in conjunction with the normal/reduced metabolic rate, these data suggest that there is increased energy use for anabolic processes.

The ability to deposit triglycerides in adipose tissue is due to having a positive energy balance. Because of its inaccessible nature, lysosomal storage does not contribute to metabolism except as an energy sink. Early in the disease process, the percentage of energy devoted to managing lysosomal storage is small. Animals with a LSD could have a positive energy balance, albeit reduced compared normal animals because of the increased de novo synthesis of raw material no longer being recycled. However, over time, the percentage of available energy devoted to managing lysosomal storage will continue to increase. In response, it is likely that the body would further compensate as if it were suffering from malabsorption or reduced caloric ingestion by increasing food intake and/or decreasing its metabolic rate. Another response could be to increase the rate of autophagy in severely affected cells, further exacerbating the problem by sequestering yet more material in
the lysosome in a feedback mechanism. In fact, an increase in autophagy has been reported recently in a mouse model of the LSD juvenile neuronal ceroid lipofuscinosis (18) and has been proposed in INCL mice (19). This relentless diversion of an increasing percentage of available nutrients to managing lysosomal storage will eventually be beyond the body’s ability to adapt. At some point, the energy available for normal cellular functions will be below the necessary level to sustain life.

In conclusion, this mechanism suggests that decreased adiposity will be a universal feature of LSD. Furthermore, if storage of material that is inaccessible to the body inevitably leads to a reduction in adiposity, then potentially any disorder with significant accumulation of irretrievable material might exhibit reduced adiposity. If true, this could represent a new form of fat loss, one whose energy is retained in an inaccessible form or compartment in the body. These findings add potentially an entire class of disorders (>40 LSDs) to the number of lean mouse models (13) available for study. In addition, these data demonstrate the importance of lysosomal recycling for the efficient utilization of energy. Finally, the mechanism described here would on its own eventually prove lethal. More important, however, increased nutrient-rich calorie consumption might delay the complications associated with lysosomal storage, including its lethality.

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REFERENCES
1. Vellodi, A. (2005) Br. J. Haematol. 128, 413–431
2. Cuervo, A. (2004) Mol. Cell. Biochem. 263, 55–72
3. Birkenmeier, E., Davison, M., Beaver, W., Ganschow, R., Vogler, C., Guy, N., Lyford, K., Maltais, L., and Wawrzyniak, C. (1989) J. Clin. Invest. 83, 1258–1266
4. Frisella, W., O’Conner, L., Vogler, C., Roberts, M., Walkley, S., Levy, B., Daly, T., and Sands, M. (2001) Mol. Ther. 3, 351–358
5. Beamer, W., and Coleman, D. (1982) Mouse Newsletter 67, 21
6. Sands, M., and Birkenmeier, E. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6567–6571
7. Du, H., Heur, M., Duanmu, M., Grabowski, G., Hui, D., Witte, D., and Mishra, J. (2001) J. Lipid Res. 42, 489–500
8. Du, H., Duanmu, M., Witte, D., and Grabowski, G. (1998) Human Mol. Gen. 7, 1347–1354
9. Du, H., Dardzinski, B., O’Brien, K., and Donnelly, L. (2005) Am. J. Roentgenol. 184, 658–662
10. Pietrobelli, A., Formica, C., Wang, Z., and Heymsfield, S. (1996) Am. J. Physiol. 271, E941–E951
11. Bernal-Mizrachi, C., Weng, S., Li, B., Nolte, L., Feng, C., Coleman, T., Holloszy, J., and Semenkovich, C. (2002) Arterioscler. Thromb. Vasc. Biol. 22, 961–968
12. Bijvelds, M., Bronsveld, I., Havinga, R., Sinaasappel, M., de Jonge, H., and Verkade, H. (2005) Am. J. Physiol. 288, G646–G653
13. Reitman, M. (2002) Annu. Rev. Nutr. 22, 459–482
14. Sartipy, P., and Loskutoff, D. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 7265–7270
15. Gerhardt, C., Romero, I., Canello, R., Camoin, L., and Strosberg, A. (2001) Mol. Cell. Endocrinol. 175, 81–92
16. Scriver, C., Beaudet, A., Sly, W., and Valle, D. (2001) The Metabolic and Molecular Bases of Inherited Disease, 8th Ed., pp. 3371–3894, McGraw-Hill Book Co., New York
17. Sands, M., Vogler, C., Kyle, J., Grubb, J., Levy, B., Galvin, N., Sly, W., and Birkenmeier, E. (1994) J. Clin. Invest. 93, 2324–2331
18. Cao, Y., Espinola, J. A., Fossale, E., Massey, A. C., Cervo, A. M., MacDonald, M. E., and Cotman, S. L. (2006) J. Biol. Chem. 281, 20483–20493
19. Hofmann, S., Lee, L., Lu, J., and Verkruyse, L. (1997) Neuropediatrics 28, 27–30