Abdominal Irradiation Ameliorates Obesity in ob/ob Mice

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Summary  Leptin-deficient ob/ob mice are a murine model for obesity, insulin resistance, and diabetes. Here we report that non-lethal abdominal irradiation (a single fraction of 850 cGy) to ob/ob mice retarded rapid gain of body weight, leading to amelioration of obesity without marked changes in food intake. This effect was observed only in ob/ob mice and not in lean controls. Reduction of body weight was accompanied by decreased adipose tissue weight without any marked change in the size of adipocytes, indicating prevention of hyperplasia rather than hypertrophy. Gene expression of the radiation-inducible cdk-inhibitor, p21, and the adipocytokines, tumor necrosis factor α and interleukin-1β, were induced as expected; but genes involved in adipogenesis such as peroxisome proliferator-activated receptor γ and adipsin were not affected in the irradiated adipose tissue. Inversely, hepatic lipid content was elevated with concomitant increases in the expression of lipogenic enzymes such as fatty acid synthase (FAS), and sterol regulatory element-binding protein 1c. Despite the decreased adiposity, there was no improvement in hyperglycemia and hyperinsulinemia after the irradiation. In conclusion, abdominal irradiation to ob/ob mice affected the progression of obesity and altered the energy metabolism between organs through a novel mechanism, implicating a new approach or factor for understanding and treatment of obesity.

Key Words: obesity, irradiation, radiation, ob/ob mice, leptin

Introduction

Obesity occurs when energy intake exceeds energy expenditure. Leptin is an adipocyte-derived hormone that plays a central role in the regulation of energy metabolism in the body [1, 2]. Leptin-deficient ob/ob mice are a model

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Abbreviations: TNF, tumor necrosis factor; IL, interleukin; SREBP, sterol regulatory element-binding protein; PPAR, peroxisome proliferator-activated receptor; TG, triglycerides; NEFA, non-esterified fatty acids; DEXA, dual-energy X-ray absorptiometry.

for obesity, insulin resistance, diabetes, and nonalcoholic steatohepatitis [3]. In ob/ob mice, the absence of the leptin signal in the hypothalamus causes obesity due to increase in food intake and decrease in energy expenditure. This is accompanied by hypertrophic and hyperplastic changes of adipocytes within white adipose tissue, and lipid accumulation in the liver. The increased adiposity is associated with insulin resistance with marked hyperinsulinemia, ultimately resulting in diabetes. Recently, pro-inflammatory proteins secreted from adipose tissue, often referred as adipokines, such as tumor necrosis factor α (TNFα) have been shown to be involved in the development of metabolic syndrome and cardiovascular risks [4, 5]. In addition, infiltration of macrophages into the fat and their interaction with the adipocytes is involved in the adipose tissue-initiated inflammation and
insulin resistance [6, 7].

Seeking for a new therapeutic approach to obesity, we have been exploring the potential impact of bone marrow-derived cells containing mesenchymal stem cells on energy metabolism in the whole body. Bone marrow transplantation caused consistent amelioration of obesity in ob/ob mice, but no change in the body weight of normal mice. Intriguingly, it was found that the irradiation to the body given as a pretreatment for the transplantation was responsible for the inhibition of body weight gain in ob/ob mice (unpublished data, Kobayashi K and Shimano H). In the current study, we investigated the specific effects of radiation on body weight and adiposity in ob/ob and wild-type mice.

Materials and Methods

Materials

All reagents were obtained from Sigma Chemicals. Enhanced chemiluminescence western blot detection kit and redivue [α-32P] dCTP were purchased from Amersham Pharmacia. Standard molecular methods were used.

Animals

The animal studies were approved by the Animal Care Committee of the University of Tsukuba. ob/ob mice (B6.V-Lepob homo, background strain: C57BL/6J) were obtained from the Jackson Laboratory, MA, and bred in the colony. Control C57BL/6 mice and littermates of B6.V-Lep+/+ were purchased from Charles River Laboratories. The mice were housed in colony cages and maintained on a 12-h light/12-h dark cycle and given free access to water and standard chow diet (MF; Oriental Yeast, Tokyo, Japan).

Abdominal irradiation

A single fraction of 850 cGy was delivered to the abdominal region of ob/ob and control mice using a gamma cell. The head and lower limbs were protected from radiation using shield lead blocks. This protocol was established as follows. Based on our initial finding that total body irradiation used for bone marrow transplantation causes suppression of weight gain in ob/ob mice, we investigated the dose-dependency and body-subfractional differences in this radiation effect. It was found that a single fraction of 850 cGy to the abdomen of db/db mice was non-lethal and did not require bone marrow transplantation for survival, but had an obesity-inhibiting potential comparable to the total body irradiation (850 cGy) with the bone marrow transplantation at 18 weeks of age.

Measurement of the biochemical data

Blood was collected from the retro-orbital venous plexus in mice fed ad libitum. The concentrations of plasma glucose, insulin, triglycerides (TG), cholesterol, and nonesterified fatty acids (NEFA) were measured using the following kits and a multilabel counter, ARVO SX 1420 (Perkin Elmer, MA).

Glucose: Glucose CII-test WAKO; Wako Pure Chemical Industries, Japan.
Insulin: Mouse Insulin ELISA (TMB) Kit; Shibayagi Co. Ltd., Japan.
Total cholesterol: Cholesterol C-test WAKO; Wako Pure Chemical Industries, Japan.
TG: Triglyceride G-test WAKO; Wako Pure Chemical Industries, Japan.
NEFA: NEFA C-test WAKO; Wako Pure Chemical Industries, Japan.
Measurement of liver TG and cholesterol was as previously described [8].

Measurement of food intake

Food intake was measured for 5 days after the mice had been adapted to individual housing for 1 week.

Measurement of body composition

Fat mass and lean tissue mass were determined using dual-energy X-ray absorptiometry (DEXA) (PIXImus; Lunar Corporation, Madison, WI) as previously described [9].

Northern blot analysis

Total RNA was extracted from the liver (10 µg) and adipose tissue (5 µg) of the mice and subjected to Northern blot analysis as previously described [8]. cDNA probes used were as previously described [10, 11].

Statistical evaluation

Two-tailed Student’s t test was used for statistical evaluation.

Results

Inhibitory effects of abdominal irradiation on body weight gain of ob/ob mice

Both male and female ob/ob mice gain body weight and adiposity more markedly and rapidly than their wild-type lean littermates (Fig. 1). This leptin deficiency-mediated acceleration of body weight gain was markedly retarded by irradiation (a single fraction of 850 cGy) in ob/ob mice of both sexes at 9 weeks of age (Fig. 1A and B). The inhibition of body weight gain became obvious approximately 8–10 weeks after the irradiation, suggesting that it was not due to acute radiation side-effects. Reduction of body weight gain by the irradiation was more prominent in female ob/ob mice than in males. In contrast, wild-type C57BL6 mice of both sexes did not show any significant percentage body weight change after a single irradiation with the same dose throughout the observation period of up to 20 weeks.
Amelioration of Obesity by Irradiation

The radiation effect was also tested at a later age (15 weeks), and a similar suppression of body weight gain was found only in ob/ob mice (data not shown).

Food intake was measured in both wild-type and ob/ob mice at 7 and 9 weeks after the radiation (Fig. 2). Unlike other manipulations to reduce the body weight of obese mice, abdominal irradiation did not significantly change their food intake. The calculated feeding efficiency showed a decreasing trend (data not shown). These data indicate that the suppressed body weight gain could not be explained by reduced ingestion of energy due to chronic side-effects on the gastrointestinal system.

Analysis of total body adipose tissue by DEXA revealed that the loss of body weight was attributed to a decrease in fat weight, whereas lean mass weight was not changed (Fig. 3). The gross appearance and anatomical inspection of the animals suggested that the decreased adiposity was proportional throughout the body and not restricted to the irradiated abdominal region (data not shown).

Effects of irradiation on metabolic parameters

Plasma metabolic parameters were measured at several time points during the growth curve. Plasma glucose, TG, and cholesterol levels were higher in ob/ob diabetic mice compared with wild-type mice; however, abdominal irradiation had no significant effects on these parameters (Table 1).

Plasma insulin levels were markedly elevated in ob/ob mice because of severe insulin resistance. There was a decreasing trend in plasma insulin level in irradiated male ob/ob mice compared with the non-irradiated control group. Thus, improvement of insulin resistance abdominal irradiation as estimated by plasma parameters was less prominent than expected from the loss of body weight.

Effects of irradiation on liver, adipose tissue, and gene expression

Three or 6 months after the irradiation, the animals were killed in a 12-h fasted state. There was a slight decrease in the weights of abdominal adipose tissues such as the epididymal fat pads and parametric fat pads in the irradiated ob/ob mice compared with the non-irradiated controls (Table 2). Conversely,
there was a slight trend of enlargement of the liver on irradiation. Histological examination demonstrated that there were no significant differences in the size or appearance of adipocytes in adipose tissue between the irradiated and non-irradiated groups (Fig. 4A). Infiltration of macrophages is known to be observed in the increased adipose tissue of obese mice including \( \text{ob/ob} \), and these macrophages contribute to pro-inflammatory responses and to insulin resistance \([6, 12]\). The infiltration of macrophages was slightly enhanced by the irradiation (Fig. 4A). Irradiation did not cause significant histological changes in the small intestine of wild-type and \( \text{ob/ob} \) mice (Fig. 4B). Hepatic lipid accumulation, as an indication of hepatosteatosis, increased slightly in the irradiated group, as visualized by an increase in both number and size of lipid droplets (Fig. 5). There was a consistent and significant increase in the hepatic TG content, but not in the cholesterol content by irradiation (Table 2). Northern blot analysis showed that hepatic expression of sterol regulatory element-binding protein-1c (SREBP-1c) and its lipogenic gene targets such as fatty acid synthase \([13, 14]\), as well as peroxisome proliferator-activated receptor \( \gamma \) (PPAR\( \gamma \)) increased, suggesting that the already activated hepatic lipogenesis in \( \text{ob/ob} \) mice was further enhanced by abdominal irradiation (Fig. 6). Expression of PPAR\( \alpha \), a well-known regulator of lipid degradation, was not changed. In adipose tissues, expression of PPAR\( \gamma \) and adipsin, markers of adipocyte differentiation were not changed by irradiation. Radiation and other cellular stresses are known to arrest cell growth at G1 by activating p21, a cyclin-dependent kinase inhibitor and a known p53 target \([15, 16]\). Recently, we reported activation of p21 in both liver and adipose tissues of \( \text{ob/ob} \) mice \([17, 18]\). Expression of p21 was further induced in both liver and adipose tissue by abdominal irradiation. TNF\( \alpha \) along with interleukin-1\( \beta \) (IL-1\( \beta \)) is believed to be involved in the adipokine hypothesis \([4]\) and is induced by irradiation.

**Discussion**

Our current study clearly demonstrates that abdominal irradiation retards the body weight gain of \( \text{ob/ob} \) mice and ameliorates obesity. We conclude that this protection is not simply due to acute radiation injury to the gastrointestinal tract. The reasons are as follows: 1) there was no significant change in food intake nor diarrhea indicating malabsorption (data not shown); 2) body weight loss became prominent 8 weeks after irradiation; and 3) body weight loss by abdominal irradiation was observed only in \( \text{ob/ob} \) mice and not in wild-type mice.

There are numerous possible mechanisms that could account for this protection from weight gain. It is possible that chronic gastrointestinal injury could be involved; however, no gross histological changes were detected in the intestine of irradiated mice (Fig. 4B). In addition, induction or suppression of hormonal factors from irradiated abdominal organs could cause body weight loss. First, cortisol deficiency from adrenal insufficiency is a possible mechanism by which weight gain in \( \text{ob/ob} \) mice could be suppressed, as adrenalectomy has been reported to ameliorate the obesity phenotypes of \( \text{ob/ob} \) mice \([19]\). However, reversal of pre-obesity by removal of the adrenal glands was associated with a decrease in food intake and plasma insulin level, neither of which was observed in the abdominally irradiated \( \text{ob/ob} \) mice \([20–23]\). Second, TNF\( \alpha \) could be involved in the current findings because it has a cachectic action by activation of lipolysis. Expression of TNF\( \alpha \) is activated in \( \text{ob/ob} \) adipose tissue, as previously reported, and is further enhanced after irradiation. However, the absence of cachectic effects in irradiated lean normal mice makes these two factors less likely to be the main cause. Despite the amelioration of obesity, irradiated \( \text{ob/ob} \) mice showed

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**Fig. 2.** Food intake of irradiated (Abd-Rad) and non-irradiated (Non-Rad) \( \text{ob/ob} \) mice after irradiation. Food intake was evaluated by measuring chow, 7 and 9 weeks after the irradiation \((n = 6–8)\).
sustained hyperinsulinemia and hyperglycemia. Food intake was not changed, and thus energy balance was not improved. However, activation of TNFα could contribute to peripheral insulin resistance [4]. Activation of adipokines such as TNFα and IL-1β could explain the sustained insulin resistance despite the marked amelioration of obesity.

DEXA revealed that weight gain was suppressed through decreased adiposity, indicating an effect of abdominal irradiation on the progress of adipose tissue to obesity in ob/ob mice. Obesity generally consists of both hypertrophy and hyperplasia of adipocytes. Decreased adiposity with no change in the size of adipocytes indicated that abdominal

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**Table 1. Serum parameters of irradiated (Abd-Rad) and non-irradiated (Non-Rad) male and female ob/ob and wild type (WT) mice fed ad libitum**

|          | WT male |        | WT female |        | ob/ob male |        | ob/ob female |        |
|----------|---------|--------|-----------|--------|------------|--------|--------------|--------|
|          | Non-Rad | Abd-Rad| Non-Rad   | Abd-Rad| Non-Rad    | Abd-Rad| Non-Rad      | Abd-Rad|
| n        | 13      | 14     | 15        | 15     | 23         | 18     | 29           | 30     |
| insulin (pg/ml) | 929 ± 146 | 1510 ± 192 | 299 ± 82.8 | 834 ± 379 | 62300 ± 15400 | 47800 ± 18200 | 40000 ± 11600 | 40900 ± 5140 |
| glucose (mg/dl)  | 265 ± 15.6 | 270 ± 18.1  | 204 ± 9.9 | 206 ± 11.8 | 225 ± 14.8 | 227 ± 15.4  | 238 ± 12.2 | 250 ± 10.3 |
| T. Chol (mg/dl)  | 113 ± 4.4 | 109 ± 4.1   | 93.1 ± 6.6 | 87.7 ± 4.2 | 239 ± 13.1 | 230 ± 12.1 | 190 ± 10.1 | 205 ± 8.6 |
| TG (mg/dl)      | 177 ± 16.0 | 165 ± 15.1  | 127 ± 29.4 | 104 ± 7.9 | 161 ± 8.9  | 175 ± 15.2 | 119 ± 5.7 | 119 ± 5.4 |
| NEFA (mEq/l)    | 0.69 ± 0.04 | 0.68 ± 0.05 | 0.69 ± 0.02 | 0.70 ± 0.11 | 0.58 ± 0.03 | 0.69 ± 0.04 | 0.64 ± 0.03 | 0.73 ± 0.07 |

T. Chol: total cholesterol, TG: triglycerides, and NEFA: non-esterified fatty acids. Values are expressed as mean ± SEM.
irradiation inhibits hyperplasia rather than hypertrophy of ob/ob adipocytes. p21, a potent cdk inhibitor, was highly induced in ob/ob adipocytes \[17\]. It is also reported to be induced at the time of clonal expansion in the differentiation of 3T3-L1 fibroblasts to adipocytes \[24\]. These data implicate the potential involvement of p21 in adipogenesis and obesity. Enhanced induction of p21 in the adipose tissue supports the concept that impairment of cell growth was involved in the decreased adiposity of irradiated ob/ob mice.

It is known that hepatocytes of ob/ob mice are hyperplastic and prone to hepatocellular carcinoma \[25\]. By extending the observation period to more than 40 weeks after the irradiation, hepatic tumors were found with a slightly higher incidence in ob/ob mice than in wild-type mice. Thus,

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**Table 2. Organ and tissue weight of irradiated (Abd-Rad) and non-irradiated (Non-Rad) male and female ob/ob and wild type (WT) mice 3 months and 6 months after the irradiation**

|                     | WT male | WT female | ob/ob Male | ob/ob Female |
|---------------------|---------|-----------|------------|--------------|
|                     | Non-Rad| Abd-Rad   | Non-Rad    | Abd-Rad      |
| Body weight (g)     | 9       | 9         | 5          | 4            |
| Liver weight (g)    | 3.05    | 1.12      | 3.96       | 3.80         |
| Paragonadal WAT (g) | 0.66±0.13 | 0.54±0.05 | 0.30±0.09  | 0.28±0.08    |
| Liver TG (mg/g)     | 79.9±11.9 | 77.8±11.7 | 68.5±21.1  | 167±13.5     |

Values are expressed as mean ± SEM.
Asterisks indicate significant differences between non-radiated mice and abdominal radiated mice (**p < 0.01, *p < 0.05).
it is possible that the cachectic action of radiation could predispose ob/ob mice to hepatic tumors [26].

Amelioration of obesity without decreased food intake led us to speculate on the fate of dietary energy in ob/ob mice after abdominal irradiation, which is currently unknown. In a reciprocal response to decreased adiposity, hepatic lipid content increased with a slight increase in lipogenesis by SREBP-1c. Abdominal irradiation could cause a shift of energy from adipose tissue to the liver as the secondary reservoir for excess energy. In addition to radiation injury, abdominal irradiation seems to contribute to insulin resistance by pro-inflammatory activation. Thus, abdominal irradiation itself is unlikely to be a future therapeutic tool against obesity. However, further studies on the precise mechanism by which irradiation works against weight gain may reveal a new factor or process that could help in the understanding of adipose tissue expansion.

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References

[1] Ingalls, A.M., Dickie, M.M., and Snell, G.D.: Obese, a new mutation in the mouse. J. Hered., 41, 317–318, 1950.
[2] Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J.M.: Positional cloning of the mouse obese gene and its human homologue. Nature, 372, 425–432, 1994.
[3] Shioita, G. and Tsuchiya, H.: Pathophysiology of NASH: insulin resistance, free fatty acids and oxidative stress. J. Clin. Biochem. Nutr., 38, 127–132, 2006.
[4] Hotamisligil, G.S., Shargill, N.S., and Spiegelman, B.M.: Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science, 259, 87–91, 1993.
[5] Spiegelman, B.M. and Flier, J.S.: Adipogenesis and obesity: rounding out the big picture. Cell, 87, 377–389, 1996.
[6] Xu, H., Barnes, G.T., Yang, Q., Tan, G., Yang, D., Chou, C.J., Sole, J., Nichols, A., Ross, J.S., Tartaglia, L.A., and Chen, H.: Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J. Clin. Invest., 112, 1821–1830, 2003.
[7] Wellen, K.E. and Hotamisligil, G.S.: Obesity-induced inflammatory changes in adipose tissue. J. Clin. Invest., 112, 1785–1788, 2003.
[8] Nakagawa, Y., Shimano, H., Yoshikawa, T., Ide, T., Tamura, M., Furusawa, M., Yamamoto, T., Inoue, N., Matsuzaka, T., Takahashi, A., Hasty, A.H., Suzuki, H., Sone, H., Toyoshima, H., Yahagi, N., and Yamada, N.: Tissue-specific transcriptional activation of hepatic IRS-2, participates in insulin signaling and ameliorates diabetes. Nat. Med., 10, 112–113, 2006.
[9] Nagy, T.R. and Clair, A.L.: Precision and accuracy of dual-energy X-ray absorptiometry for determining in vivo body composition of mice. Obes. Res., 8, 392–398, 2000.
[10] Inoue, N., Shimano, H., Nakakuki, M., Matsuzaka, T., Nakagawa, Y., Yamamoto, T., Sato, R., Takahashi, A., Sone, H., Yahagi, N., Suzuki, H., Toyoshima, H., and Yamada, N.: Lipid synthetic transcription factor SREBP-1a activates p21WAF1/CIP1, a universal cyclin-dependent kinase inhibitor. Mol. Cell Biol., 25, 8938–8947, 2005.
[11] Matsuzaka, T., Shimano, H., Yahagi, N., Amemiya-Kudo, M., Okazaki, H., Tamura, Y., Iizuka, Y., Ohashi, K., Tomita, S., Sekiya, M., Hasty, A., Nakagawa, Y., Sone, H., Toyoshima, H., Ishibashi, S., Osuga, J., and Yamada, N.: Insulin-independent induction of sterol regulatory element-binding protein-1c expression in the livers of streptozotocin-treated mice. Diabetes, 53, 560–569, 2004.
[12] Weisberg, S.P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R.L., and Ferrante, A.W., Jr.: Obesity is associated with macrophage accumulation in adipose tissue. J. Clin. Invest., 112, 1796–1808, 2003.
[13] Shimano, H., Yahagi, N., Amemiya-Kudo, M., Hasty, A.H., Osuga, J., Tamura, Y., Shionoiri, F., Iizuka, Y., Ohashi, K., Harada, K., Gotoda, T., Ishibashi, S., and Yamada, N.: Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. J. Biol. Chem., 274, 35832–35839, 1999.
[14] Shimano, H.: Sterol regulatory element-binding protein family.
as global regulators of lipid synthetic genes in energy metabolism. *Vitam. Horm.*, 65, 167–194, 2002.

[15] el-Deiry, W.S., Tokino, T., Velculescu, V.E., Levy, D.B., Parsons, R., Trent, J.M., Lin, D., Mercer, W.E., Kinzler, K.W., and Vogelstein, B.: WAF1, a potential mediator of p53 tumor suppression. *Cell*, 75, 817–825, 1993.

[16] Harper, J.W., Adami, G.R., Wei, N., Keyomarsi, K., and Elledge, S.J.: The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell*, 75, 805–816, 1993.

[17] Yahagi, N., Shimano, H., Matsuzaka, T., Najima, Y., Sekiya, M., Nakagawa, Y., Ide, T., Tomita, S., Okazaki, H., Tamura, Y., Iizuka, Y., Ohashi, K., Gotoda, T., Nagai, R., Kimura, S., Ishibashi, S., Osuga, J., and Yamada, N.: p53 Activation in adipocytes of obese mice. *J. Biol. Chem.*, 278, 25395–25400, 2003.

[18] Yahagi, N., Shimano, H., Matsuzaka, T., Sekiya, M., Najima, Y., Okazaki, S., Okazaki, H., Tamura, Y., Iizuka, Y., Inoue, N., Nakagawa, Y., Takeuchi, Y., Ohashi, K., Harada, K., Gotoda, T., Nagai, R., Kadowaki, T., Ishibashi, S., Osuga, J., and Yamada, N.: p53 involvement in the pathogenesis of fatty liver disease. *J. Biol. Chem.*, 279, 20571–20575, 2004.

[19] Solomon, J., Bradwin, G., Cocchini, M.A., Coffey, D., Condon, T., Garrity, W., and Grieco, W.: Effects of adrenalectomy on body weight and hyperglycemia in five months old Ob/Ob mice. *Horm. Metab. Res.*, 9, 152–156, 1977.

[20] Wittmers, L.E.Jr. and Haller, E.W.: Effect of adrenalectomy on the metabolism of glucose in obese (C57 Bl/6J ob/ob) mice. *Metabolism*, 32, 1093–1100, 1983.

[21] Ohshima, K., Shargill, N.S., Chan, T.M., and Bray, G.A.: Adrenalectomy reverses insulin resistance in muscle from obese (ob/ob) mice. *Am. J. Physiol.*, 246, E193–197, 1984.

[22] Saito, M. and Bray, G.A.: Adrenalectomy and food restriction in the genetically obese (ob/ob) mouse. *Am. J. Physiol.*, 246, R20–25, 1984.

[23] Feldkircher, K.M., Mistry, A.M., and Romsos, D.R.: Adrenalectomy reverses pre-existing obesity in adult genetically obese (ob/ob) mice. *Int. J. Obes. Relat. Metab. Disord.*, 20, 232–235, 1996.

[24] Nakae, J., Kitamura, T., Kitamura, Y., Biggs, W.H., 3rd, Arden, K.C., and Accili, D.: The forkhead transcription factor Foxo1 regulates adipocyte differentiation. *Dev. Cell*, 4, 119–129, 2003.

[25] Yang, S., Lin, H.Z., Hwang, J., Chacko, V.P., and Diehl, A.M.: Hepatic hyperplasia in noncirrhotic fatty livers: is obesity-related hepatic steatosis a premalignant condition? *Cancer Res.*, 61, 5016–5023, 2001.

[26] Thompson, C.I., Kreider, J.W., and Margules, D.L.: Food intake during tumor growth: anorexia in genetically obese ob/ob mice and hyperphagia in lean mice. *Physiol. Behav.*, 32, 935–939, 1984.