Ovariectomy in Mice Decreases Lipid Metabolism-Related Gene Expression in Adipose Tissue and Skeletal Muscle with Increased Body Fat

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(Received October 21, 2004)

Summary Postmenopausal women as well as rodents after ovariectomy, which results in a lack of estrogen, can become obese. Ovariectomy-induced obesity in mice is associated with a decrease in oxygen consumption, indicating repressed energy expenditure. In this study, to elucidate the mechanism of weight gain after ovariectomy, we examined the expression patterns of genes related to energy expenditure and lipid metabolism, in mouse tissues including adipose tissue and skeletal muscle. In adipose tissue and skeletal muscle, at 2–4 wk after ovariectomy, levels of nuclear receptors and co-factors involved in energy expenditure such as ERR1, PPARα and PPARγ, and PGC1α and PGC1β were lower than in control mice. mRNA levels of their targets, medium-chain acyl coenzyme A dehydrogenase and acetyl CoA oxidase, enzymes for fatty acid β-oxidation, were lower. In addition, the expression of PPARγ and SREBP1, transcription factors important for lipogenesis, was decreased, as well as that of acetyl CoA carboxylase and fatty acid synthase, enzymes for fatty acid synthesis, and diacylglycerol acyl transferase 1 and 2. These changes in gene expression are consistent with the obese phenotype in mice after ovariectomy. Thus a decrease in the expression of energy expenditure-related genes in adipose tissue and skeletal muscle could, in part, be responsible for obesity after ovariectomy.

Key Words obesity, estradiol, energy, fat, ovariectomy

Ovariectomy (OVX), which results in a lack of estrogen, causes obesity in mice (1). The amount of white adipose tissue (WAT) is increased by OVX and decreased by estrogen. Similar observations have been made in postmenopausal women on hormone replacement therapy. A deficiency in estradiol in rodents is associated with a decrease in oxygen consumption, indicating repressed energy expenditure (2). Most, but not all, effects of estrogen are considered to be mediated by estrogen receptors (ER), a family of nuclear hormone receptor-type transcription factors. ERα knockout mice have decreased energy expenditure and become obese (2). However, the precise nature of this decrease in energy expenditure is unknown.

Estrogen receptor related receptors (ERRs) belong to the nuclear receptor family. ERRs are highly similar to ERs. ERR1 is prominently expressed in tissues with a high capacity for fatty acid β-oxidation such as skeletal muscle and brown adipose tissue (BAT), suggesting that it plays a role in regulating the cellular energy balance (3). This is further supported by the evidence that ERR1 is a regulator of the medium-chain acyl coenzyme A dehydrogenase (MCAD) gene (4, 5), in combination with PGC-1α and/or PGC-1β, co-activators of nuclear receptors including ERR1 (6–9).

As well as ERRs, several nuclear receptors and transcription factors are considered to be involved in energy expenditure and lipid metabolism. PPARs also belong to the nuclear receptor family. PPARα is activated by various compounds including fibrate and n-3 fatty acids that stimulate β-oxidation of fatty acid in liver (10). PPARγ is a potent activator of adipose differentiation (11). The activation of PPARγ in adipose tissue (12) and skeletal muscle (13, 14) leads to an activation of energy expenditure and resistance to obesity. On the other hand, SREBP1 is another important transcription factor that regulates fatty acid metabolism in liver and skeletal muscle (15).

In this study, to elucidate the mechanism of weight gain after OVX, we examined the expression patterns of genes related to energy expenditure and lipid metabolism, in tissues including adipose tissue, skeletal muscle and liver. The results indicated that a decrease in the expression levels of energy expenditure-related genes in adipose tissue and skeletal muscle and an increase in lipid synthesis in liver might be responsible for obesity after OVX.

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MATERIALS AND METHODS

Animals. Female C57BL/6J mice obtained from Tokyo Laboratory Animals Science (Tokyo, Japan) were ovariectomized or sham-operated under pentobarbital anesthesia using a midline incision. In experiment 2, female ddY mice were ovariectomized. The mice were individually housed in a temperature (22°C) and light (12/12 h light-dark cycle)-controlled environment. They had free access to a standard chow (CE2, Clea Japan Inc., Tokyo) and water. All procedures were in accordance with institutional guidelines.

Body composition analysis. Mice were anesthetized with pentobarbital sodium. Nembutal (0.08 mg/g body weight, Abbot Laboratories, Chicago, IL), and scanned with a Lunar PIXI mus2 densitometer (Lunar Corporation, Madison, WI), equipped for dual energy X-ray absorptiometry (DEXA).

Blood analysis. Blood samples were obtained from the tail tip for hormone and metabolite determination under fed conditions. Free fatty acid content was measured with a NEFA C-test Wako (Wako, Osaka, Japan) and glucose by using a TIDEX glucose analyzer (Sanka, Tokyo, Japan).

RNA preparation and Northern-blot analysis. Total RNA was prepared from tissue with TRizol (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer’s instructions. The cDNA fragments for probes were obtained by RT-PCR and confirmed by sequencing. Northern blotting was performed as described elsewhere (16).

Western-blots analysis. Protein samples were prepared and Western-blotting performed as described previously (16). Anti-PGClα (Calbiochem) and anti-MCAD (Cayman) were used as primary antibodies (1:1,000).

Statistical analyses. Statistical comparisons of data were made with a one-way analysis of variance (ANOVA), and each group was compared with the others using Fisher’s protected least significant difference (PLSD) test (Statview 4.0, Abacus Concepts, Piscataway, NJ). Statistical significance was defined as p<0.05.

RESULTS

OVX-induced obesity

Sixteen mice were ovariectomized (OVX mice) and another 12 were sham-operated (control mice). Half of these animals were used for the following analysis 2 wk after surgery and the other half at 4 wk after surgery. Body weight, weights of uteri, fat, and skeletal muscle, body fat content, and blood levels of triglyceride, glucose and fatty acids were examined (Table 1).

Body weight was significantly higher in the OVX mice than in the control mice at both 2 and 4 wk after surgery. Uterine weight decreased markedly in the OVX mice, indicating that the animals were deficient in estrogen. The body composition was determined by DEXA and dissection of the adipose tissue. DEXA analysis showed that the amount of adipose tissue was markedly increased in the OVX mice. 4 wk after surgery.

| Table 1. Body weight, tissue weight, and serum metabolite levels of OVX and sham-operated mice. |
|---|---|---|---|---|---|---|
| | Body weight of mice (g) | Body weight of uterine (g) | WAT (g) | Liver (g) | Muscle (g) | Body fat content (%) |
| 2 wk Sham | 21.5±0.36 | 0.10±0.03 | 0.26±0.03 | 0.20±0.01 | 0.20±0.01 | 0.27±0.03 |
| OVX | 22.9±0.57 | 0.10±0.03 | 0.26±0.03 | 0.20±0.01 | 0.20±0.01 | 0.27±0.03 |
| 4 wk Sham | 24.0±0.74 | 0.21±0.03 | 0.28±0.10 | 0.22±0.02 | 0.24±0.03 | 0.31±0.05 |
| OVX | 27.8±0.55 | 0.41±0.03 | 0.30±0.07 | 0.34±0.08 | 0.34±0.07 | 0.34±0.07 |

Mice were used for analysis at 2 and 4 wk after surgery. Marked uterine hypoplasia was obvious in OVX but not sham-operated mice. Statistical comparisons were made between OVX and sham-operated mice at the same time points after surgery. Each value is the mean±SE. *p<0.05; **p<0.01; ***p<0.001. 

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compared to the control mouse. The OVX mice also had a significantly increased amount of dissected parametorial WAT. Bone mineral content was significantly decreased in the OVX mice. Liver weight was increased at 2 and 4 wk after OVX, but the amount of skeletal muscle was not affected. Blood metabolites (glucose, FFA, TG), did not differ between OVX and control mice. 

**Gene expression in adipose tissues and skeletal muscle of OVX mice**

RNA samples (BAT, skeletal muscle, WAT and liver) were obtained from the mice. RNA samples for each tissue were combined and pooled in each group and used for Northern blot analysis. We performed Northern blotting using probes for lipid usage and lipid synthesis. Lipid usage-related genes examined were: ERα, ERR1, PPARα, and PPARδ for nuclear receptors, PGC1α and PGC1β for nuclear receptor cofactors. MCAD, acetyl CoA oxidase (ACO) for fatty acid β-oxidation (17). Lipid synthesis-related genes were: PPARγ for a nuclear receptor, SREBP1 for a transcription factor, acetyl CoA carboxylases (ACC1), fatty acid synthase (FAS) for fatty acid synthesis and diacyl glycerol acyl transferase (DGAT1) and DGAT2 for triglyceride synthesis (17). In BAT, considered an important tissue of energy expendi-

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**Fig. 1.** Lipid metabolism-related gene expression in BAT of OVX mice. mRNA extracted from BAT of 6 OVX mice and 8 sham-operated mice (control) at 2 or 4 wk after surgery was subjected to Northern blotting. Samples in each group were combined for analysis. The same RNA sample sets were blotted onto multiple membranes and hybridized with the probes indicated. Equal sample loading was confirmed by ethidium bromide staining of 28S ribosomal RNA (not shown). The names of genes examined are on the right of the autoradiograms, and densitometric ratios (the control, a sample of a sham-operated mice at 2 wk after surgery, was set as 100) are beneath the panel. Densitometric ratios are also shown as graphs on the right. Closed and open bars show results for OVX and control mice, respectively.
Ovariectomy in Mice Changes Lipid Metabolism-Gene Expression

Fig. 2. Lipid metabolism-related gene expression in skeletal muscle of OVX mice. Northern blots were obtained as described in the legend for Fig. 1.

ture in mice. expression levels of nuclear receptors and cofactors involved in energy expenditure such as ERR1, PPARα and PPARδ, and PGC1α and PGC1β were lower than in control mice at both week 2 and 4 (Fig. 1). mRNA levels of their targets, MCAD, an enzyme for mitochondrial fatty acid β-oxidation, and ACO, a peroxisomal fatty acid β-oxidation enzyme, were also lower. Thus, the activity for fatty acid β-oxidation in BAT may be decreased in OVX mice. In addition, the expression of PPARγ and SREBP1, transcription factors important for lipogenesis, was decreased. The expression of ACC1 and FAS, enzymes for fatty acid synthesis, and DGAT1 and DGAT2, enzymes for triglyceride synthesis, was also decreased (Fig. 1). Gene expression changes in skeletal muscle (Fig. 2), WAT (Fig. 3) and BAT for lipid metabolism were basically similar. In skeletal muscle, the decrease in expression was relatively mild compared to that in WAT and BAT. Thus, many genes involved in both lipid synthesis and energy expenditure were markedly down-regulated in adipose tissue. This is consistent with the observation that both fatty acid synthesis and fatty acid β-oxidation are decreased in WAT of obese mice (18, 19).

Gene expression in liver of OVX mice

The expression of PPARδ in the liver was markedly lower in the OVX mice than in the control mice. PPARδ is involved in the activation of β-oxidation (14), MCAD expression was also decreased in the liver of the OVX mice. The expression of lipid synthesis-related genes in the liver, such as ACC1, FAS and DGAT1, was higher 4 wk after OVX than in the control mice. The liver was heavier in the OVX mice than in the control mice (Table
WAT

2W  4W
-  + -  +

ERα
100  75  87  45
100  90  108  86
100  82  63  42

ERR1
100  73  97  52
100  81  102  68
100  71  83  41

PPARα
100  111  101  89
100  100  107  46
100  81  89  58

PPARδ
100  71  83  41
100  68  85  78
100  85  116  92

PGC1α
100  71  83  41
100  52  70  41
100  81  83  57

PGC1β
100  71  83  41
100  68  85  78
100  85  116  92

MCAD
100  71  83  41
100  68  85  78
100  85  116  92

ACO
100  71  83  41
100  68  85  78
100  85  116  92

Lipid synthesis

Lipid usage

Fig. 3. Lipid metabolism-related gene expression in WAT of OVX mice. Northern blots were obtained as described in the legend for Fig. 1.

In Figs. 1–4, we pooled RNA samples. For analysis of statistical significance for representative gene expression change, we repeated the experiments (experiment 2). Four mice were ovariectomized and another four mice sham operated (control). The mice were killed 2 wk later and used for analysis. Body weight was significantly higher in the OVX mice (average 34.0±0.48 g) than in the control mice (29.9±0.44 g, p<0.001). Uterine weight decreased markedly in OVX mice (Sham, 0.154±0.015 g; OVX, 0.020±0.004 g, p<0.001). RNA samples from BAT and skeletal muscle was obtained and used for Northern blot analysis. Using probes for the energy usage related genes PGC1α, PGC1β, ERR1 and MCAD; we found that the expression was significantly reduced in both BAT and skeletal muscle of OVX mice (Fig. 5A). Also, we examined protein levels of PGC1α and MCAD in BAT. Not only mRNA levels, but also protein levels were significantly decreased in OVX mice (Fig. 5B).

DISCUSSION

We examined changes in the expression of various nuclear receptors, cofactors and their target genes involved in lipid metabolism, in the tissues after OVX. Expression of genes related to lipid metabolism including energy expenditure in adipose tissue and muscle
was decreased, and that of genes related to lipid synthesis in the liver increased. In our preliminary experiments, in the skeletal muscle of KK-Ay mice, another type of obese mice, repressed expression of the energy expenditure-related genes, such as ERR1 and PGC1\(\beta\), was not observed (not shown). Thus, the decreased expression of energy expenditure-related genes in OVX mice is not likely the result of obesity, but is possibly a cause of obesity. Thus, a decrease in the expression levels of energy expenditure-related genes in adipose tissue and skeletal muscle and an increase in lipid synthesis in the liver might be responsible for obesity after OVX.

Expression of energy expenditure-related genes in adipose tissue and skeletal muscle was decreased after OVX. What nuclear receptors and other transcription factors are responsible for the down-regulation of gene expression after OVX? ERR1 is a regulator of the MCAD gene, in combination with PGC-1\(\alpha\) and/or PGC-1\(\beta\) (7–9). ACO gene has PPAR-responsive elements (21–23). In addition, Huss et al. reported that ERR1 activates PPAR\(\alpha\) gene expression (24). Thus, decreased expression of ERR1 and PPAR\(\alpha\), and possibly PPAR\(\delta\), appears to be responsible for the down-regulation of genes for energy expenditure. As ER\(\alpha\) knockout mice have decreased energy expenditure and become obese (2), ER\(\alpha\) may be involved in the process, but there are no reported ER-responsive elements in MCAD or ACO. Interestingly, ER\(\alpha\) and ERR1 can physically interact and cooperatively activate estrogen-dependent transcription in the lactoferrin gene (25). Thus, ER\(\alpha\), with ERR1, may be involved in gene regulation in adipose and skeletal muscle after OVX. Ovaries produce female sex hormones including estradiol and its precursor progesterone. Thus the decrease in the level of female sex
hormones after OVX could be associated with the markedly lower uterine weights in OVX mice in our experiments. Estradiol has been reported to stimulate expression of progesterone receptors, thereby affecting the responsiveness of the action of progesterone (26). The gene expression via ER, ERR, and possibly the progesterone receptor was probably lower because of the decreased level of female sex hormones.

The expression of lipid synthesis-related genes in the liver, such as ACC1, FAS and DGAT1, was higher in OVX mice than in control mice. SREBP1, a transcription factor important for lipogenesis, is known to be a regulator of ACC1 and FAS (15). However, expression of SREBP1 in the liver of OVX mice was similar to that in the control mice. In contrast, PPARγ expression in the liver was higher in the OVX mice than in the control mice. However, to our knowledge, there have been no reports that PPARγ regulates ACC1, FAS or DGAT1. Thus, transcription factor(s) other than SREBP1 and PPARγ may be involved in the up-regulation of lipid synthesis-related genes in the liver of OVX mice. This remains to be further studied.

In this study, the expression of genes related to lipid metabolism including energy expenditure in adipose tissue and muscle was decreased, and that of genes related to lipid synthesis in the liver increased. These changes are consistent with the obese phenotype in OVX rodents. Thus, these proteins may play a role in the regulation of energy metabolism in OVX-induced obesity. Further study should provide more insight into the molecular mechanism of OVX-induced obesity, which could be of medical importance.

Acknowledgments

We thank M. Fujioka for technical assistance. This work was in part supported by the Japan Health Foundation.

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