Compensation for an increase in body fat caused by donor transplants into mice

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Rooks, Cherie, TaNeisha Bennet, Timothy J. Bartness, and Ruth B. S. Harris. Compensation for an increase in body fat caused by donor transplants into mice. Am J Physiol Regul Integr Comp Physiol 286: R1149–R1155, 2004. First published February 26, 2004; 10.1152/ajpregu.00634.2003.—Rodents tend to compensate for experimental obesity in which both adipocyte size and number are increased. In contrast, it was recently reported that Siberian hamsters do not compensate for dorsal subcutaneous transplants of fat, which increase body fat without changing the size of adipocytes. In the first experiment described here we tested whether mice changed the size of their endogenous fat stores 2 or 5 wk after donor fat was added as subcutaneous transplants. Each epididymal fat pad from donor mice was cut in half and placed ventrally in recipient mice, increasing body fat by ~10%. After 2 wk, there was no effect of the transplants on the size of endogenous fat depots or the size of adipocytes in epididymal fat depots. There was a substantial decrease in mass and adipocyte size in transplanted fat. Five weeks after surgery the endogenous epididymal and retroperitoneal fat depots of recipient mice were significantly decreased, serum leptin was reduced, and the size of adipocytes in endogenous epididymal fat was significantly reduced, although cell number had not changed. The size of transplanted cells was the same as at 2 wk. In a second experiment, epididymal fat was placed as either dorsal or ventral subcutaneous fat transplants. Five weeks after surgery the endogenous fat depots were decreased in all recipient mice but none of the differences reached statistical significance. These results suggest that mice have mechanisms to maintain total body fat mass that respond to an increase in the number of fat cells present.

body weight; white adipose tissue; cellularity; carcass composition; leptin

THE LIPOSTATIC HYPOTHESIS for the regulation of body weight was proposed by Kennedy and colleagues (4, 18, 19), who suggested that calorie intake was regulated by a mechanism that included the hypothalamus to prevent “a plethora of stored fat” (4). Since the introduction of this hypothesis, a number of different models have been used to support the concept that, over the long term, energy intake is controlled to maintain the body fat mass of an individual within a narrow range. Experiments in which body weight is reduced by food restriction show that animals return to their control body composition once the intervention is removed (14). This recovery is associated with a period of overeating that supports the energy cost of compensatory growth (14). Specific reduction of body fat, independent of a reduction in the size of fat cells or a loss of lean tissue, has been achieved in lipectomy studies in which individual, or multiple, fat depots are surgically removed (for review, see Ref. 22). In these studies, the anatomical location of the pad determines whether it regenerates (24), but, regardless of the incidence of regeneration, total body fat mass is recovered over a period of months due to enlargement of the remaining fat depots. The surgically induced depletion of body fat and its reparation are not associated with a measurable hyperphagia, suggesting that the replacement of body fat is achieved through shift in nutrient partitioning and by subtle changes in energy intake and expenditure (22).

Despite the growing evidence that environmental and dietary factors can override regulatory mechanisms and lead to obesity in the human population (26), experimental animals that have been forced to gain weight by overfeeding spontaneously reduce their voluntary energy intake until control body weight is recovered once the overfeeding ends (12). Parabiosis studies, in which obese and lean rodents share a common blood supply (17), suggest that adipose-derived circulating factors, such as leptin (5, 10), play a critical role in the detection of excessive body fat and in the feedback regulatory system that responds to correct the excess. Far fewer studies have tested the regulation of body fat by directly increasing total body fat mass using transplants. Ashwell and colleagues (2, 3) reported a number of experiments in which physiologically insignificant (5–10 mg) amounts of fat were transplanted under the kidney capsule of recipient mice and demonstrated that transplanted fat acquired the characteristics of endogenous fat, indicating that environment was a more powerful determinant than origin in determining the physical characteristics of fat cells. The amount of fat transplanted in these experiments was too small to be used to investigate the regulation of body fat mass. Recently, Reitman’s (23) group developed a lipoatrophic, zinc finger-deficient mouse. Fat transplants into these mice corrected many of their metabolic disorders (9), and the experiments demonstrated that relatively large pieces of transplanted fat remain viable for a significant length of time and have a physiological impact. In addition to these observations, Lacy and Bartness (20) tested whether lipectomized Siberian hamsters showed the normal enlargement of remaining fat depots if the fat that had been lipectomized was replaced as subcutaneous transplants. The addition of epididymal fat, but not inguinal fat, as a transplant appeared to exaggerate the compensatory hypertrophy of endogenous fat depots in lipectomized hamsters. In contrast, nonlipectomized hamsters did not compensate for an increase in body fat mass produced by dorsal fat transplants, and the size of their endogenous fat depots were the same as those of animals that did not receive fat transplants.

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The objective of this study was to test whether mice that experienced an increase in fat mass caused by subcutaneous transplants of fat from donor animals were able to detect the increase and respond by reducing the size of endogenous fat stores. There was a potential for a difference in response between species, because Siberian hamsters are able to increase or decrease the size of their body fat stores according to the seasonal changes in day length. In contrast, mice and rats do not naturally exhibit large changes in body fat mass.

METHODS

Experiment 1: ventral fat transplants in National Institutes of Health Swiss mice. Fifty-four male, 5-wk-old National Institutes of Health (NIH) Swiss mice (Harlan Sprague Dawley, Indianapolis, IN) were housed individually in a temperature-controlled room at 23°C, 55% humidity, with free access to chow (Purina Mouse Chow 5001, Purina Mills, St. Louis, MO) and water and with lights on for 12 h each day from 7:00 AM. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Georgia and were conducted according to National Institutes of Health and American Physiological Society guidelines (1).

The mice were allowed to adapt to the environment for 1 wk. They were then placed in cages with grid floors and given free access to a pelleted 10% kcal fat diet (Diet D12450B: Research Diets, New Brunswick, NJ). Daily body weights and food intakes, corrected for spillage, were recorded for 10 days. At the end of this baseline period, the mice were divided into three weight-matched groups of 18 mice. One group was sham-operated controls, a second group was recipient mice, and the third group was donor mice. Surgeries were carried out with the mice under isoflurane anesthesia. Epididymal fat was carefully dissected from the donor mice, and each fat pad was weighed and cut in half. The donor mice were killed by carbon dioxide inhalation. The four pieces of fat were placed in sterile saline. Four small incisions were made in the skin of the ventral area of the recipient mice. This is an area that has a minimal amount of endogenous subcutaneous adipose tissue. Skin was freed from connective tissue using blunt dissection, and each of the four pieces of donor fat was placed subcutaneously in the recipient mice. The fat from the right side of the donor mouse was placed on the right side of the recipient mouse, and fat from the left side was placed on the left side of the recipient mouse. The incisions were sutured, and the mice were allowed to recover from anesthesia before being returned to their home cages. Sham-operated mice were anesthetized, incisions were made, and skin was separated from connective tissue but no fat was transferred. The mice within each group were separated into two weight-matched subgroups. One subgroup of eight mice from each treatment was killed 2 wk after surgery, and the remaining 10 animals in each treatment group were killed 5 wk after surgery.

On the days that mice were killed, food was removed from the cages at 7:00 AM and tissue collection started at 9:00 AM. The mice were decapitated, and trunk blood was collected for measurement of serum leptin (Mouse Leptin RIA kit; Linco Research, St Louis, MO). The transplanted fat from the right and the left side of the recipient mice, right and left epididymal fat pads, and retroperitoneal and mesenteric fat depots were dissected and weighed. The gastrointestinal tract was cleaned and returned to the carcass. A small piece (~50 mg) of the left epididymal fat pad and of the left transplant was fixed in osmium tetroxide for determination of fat cell size and number by Coulter Counter equipped with a channelizer, as described previously (13). The carcass was analyzed for composition, as described previously (15).

Statistically significant differences between groups for daily measures of food intake, body weight, and fat cell size distribution were determined by repeated-measures ANOVA and post hoc unpaired t-tests or one-way ANOVA. Changes in the weights of transplanted fat were determined by paired t-test. All other comparisons between sham-operated and recipient mice were determined by unpaired t-test assuming equal variance. Statistical analysis was carried out using Statistica software (StatSoft, Tulsa, OK).

Experiment 2: comparison between ventral and dorsal fat transplants. The previous study showed that mice that received ventral fat transplants compensated for the addition of body fat by decreasing the size of their endogenous fat depots. These results contrasted with those reported by Lacy and Bartness (20) for hamsters that did not compensate for dorsal fat transplants. Therefore, this study determined whether the difference between the mouse and the hamster studies was due to the location of the fat transplant. Two groups of recipient mice were included in the study. One group received ventral transplants as described above. The second group had the fat transplants placed dorsally, in contact with either inguinal or subcutaneous fat.

Fifty male, 5-wk-old NIH Swiss mice (Harlan Sprague Dawley) were housed as described above. After 1 wk, the mice were transferred to cages with grid floor and fed the 10% kcal fat diet described above. Body weights were recorded every second day, but food intakes were not measured because there was no effect of treatment on intake in experiment 1. After 1 wk of baseline measures, the mice were divided into five groups of 10 mice. One group was sham operated, one group received dorsal bilateral subcutaneous transplants of epididymal fat, and one group received ventral bilateral subcutaneous transplants of epididymal fat. The remaining two groups of mice donated the fat for the transplants. Body weights of the mice were recorded for 5 wk, and then the mice were killed for measurement of fat depot weights, fat cell size, and body composition, as described above. The study was ended at 5 wk because this was a time at which we found a significant response to transplants in recipient mice in experiment 1.

Statistically significant differences between groups for body weight and fat cell size distribution were determined by repeated-measures ANOVA and post hoc Duncan’s multiple-range test. Changes in the weights of transplanted fat were determined by two-way ANOVA. All other comparisons between sham-operated and recipient mice were determined by one-way ANOVA. Statistical analysis was carried out using Statistica software (StatSoft, Tulsa, OK).

RESULTS

Experiment 1. There were no significant differences in the body weights of the sham-operated and recipient mice 2 wk postoperatively. Although the recipient mice appeared to have gained more weight than the sham-operated mice, this difference was not statistically significant (see Table 1). Simultaneously, there were no differences in the cumulative food intakes of the mice during the 2 wk after surgery or in the body composition of the animals (see Table 1). Approximately 440 mg of fat was transplanted into the recipient mice and 374 mg was recovered (Fig. 1C). The transplanted fat did not produce a statistically significant increase in total carcass fat content of the two groups, and there were no differences in serum leptin concentrations (Table 1). There was no effect of the fat transplants on the size of endogenous fat depots 2 wk after surgery (Fig. 1A). In addition, there was no effect of fat transplants on the size or number of endogenous epididymal fat cells in recipient mice, although the size of the transplanted cells (treatment: P = 0.0002, size: P < 0.0001, interaction: P < 0.0001) and the total number of transplanted epididymal cells detected by the Coulter Counter were significantly smaller than those of endogenous epididymal fat (Fig. 2).
Table 1. Body weight, food intake, and carcass composition of mice, measured 2 or 5 wk after the fat transplant in experiment 1

<table>
<thead>
<tr>
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<th>2 Weeks</th>
<th>5 Weeks</th>
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<tr>
<td></td>
<td>Sham</td>
<td>Recipient</td>
</tr>
<tr>
<td>Presurgical wt, g</td>
<td>34.7±0.5</td>
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<tr>
<td>Final body wt, g</td>
<td>36.5±0.8</td>
<td>36.5±0.6</td>
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<tr>
<td>Weight change, g/14 or 35 day</td>
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<td>3.3±0.5</td>
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<tr>
<td>Food intake, g/14 or 35 day</td>
<td>66.3±1.6</td>
<td>65.1±0.9</td>
</tr>
<tr>
<td>Serum leptin, ng/ml</td>
<td>2.8±0.4</td>
<td>3.1±0.6</td>
</tr>
<tr>
<td>Carcass wt, g</td>
<td>32.8±0.9</td>
<td>32.4±0.7</td>
</tr>
<tr>
<td>Carcass fat, g</td>
<td>3.3±0.3</td>
<td>3.6±0.4</td>
</tr>
<tr>
<td>Carcass lean tissue, g</td>
<td>28.2±0.7</td>
<td>27.4±0.6</td>
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Data are means ± SE for groups of 8 or 10 mice. Recipient mice received ventral fat transplants of epididymal fat from donor mice 2 or 5 wk before determination of carcass analysis. There were no significant differences in any of the parameters measured after 2 wk. *Significant difference, at $P < 0.02$, in serum leptin concentrations between sham-operated and recipient mice 5 wk after surgery.

There were no differences in daily or cumulative food intakes of the sham-operated and transplant mice that were killed 5 wk postoperatively (Table 1 and Fig. 3). Statistical analysis indicated a significant interaction between treatment and day on body weight of the mice [treatment: not significant (NS), day: $P < 0.0001$, interaction: $P < 0.003$], but post hoc analysis did not identify any specific days on which the difference between groups reached statistical significance. Similar results were found when weight change from preoperative weight was analyzed [treatment: NS, day: $P < 0.0001$, interaction: $P < 0.0003$; data not shown]. Carcass analysis did not reveal any differences between the sham-operated and recipient mice (Table 1), but the weights of individual fat pads were significantly smaller in the recipient than sham-operated mice (Fig. 1B), and serum leptin was significantly decreased in recipient mice (Table 1). The reduction in size of the fat pads was depot specific, with the biggest effect in epididymal fat and a nonsignificant reduction in size in mesenteric fat. The weight of the transplanted fat also had significantly decreased from 500 to 351 mg during the 5 wk after surgery (Fig. 1D). The transplants had a similar number of measurable cells as were present in the 2-wk transplants (Fig. 2C) and there was no further reduction in size of the transplanted cells between 2 and 5 wk postoperatively. The decrease in size of endogenous epididymal fat pads in the recipient mice was due to a decrease in fat cell size (Fig. 2B; treatment: $P < 0.001$, size: $P < 0.0001$, interaction: $P < 0.0001$), with no change in fat cell number, compared with either the 5-wk sham-operated mice or with the two groups of mice killed 2 wk after surgery (Fig. 2C).

Experiment 2. There were no differences in the average body weights of the three groups of mice during the 5 wk after surgery (data not shown). Similarly there were no differences in the amount of weight gained during the study, the final body weights of the mice, or carcass fat at the end of the study (Table 2). The weights of individual fat pads tended to be smaller in both groups of recipient than sham-operated mice (Fig. 4A), although the differences were not significant. Serum leptin was not different between the three groups (Table 2). The weight of the transplanted fat had decreased during the 5 wk after surgery, but the difference was significant only for the ventral transplants (Fig. 4B; $P < 0.02$). There were no differences in the number of fat cells present in epididymal fat of mice in each of the three treatment groups and there was no difference in the number of cells present in the ventral or dorsal fat transplants. The number of cells present in the transplanted epididymal fat was significantly less than the number present in endogenous epididymal fat depots (Fig. 5A) and the cell size distribution of these cells was skewed toward smaller diameter cells (Fig. 5B).
DISCUSSION

The outcome of this study supports the notion that total body fat mass is regulated. These experiments contrast with other experiments that have shown the correction of an increase in body fat that is caused by overfeeding because the transplants initially increase the number of fat cells in an animal without changing the size of endogenous fat cells. In contrast, experimentally induced obesity causes an increase in both the size and number of cells in existing endogenous fat depots (12) and recovery from this increase in body fat reflects the ability of fat depots to return to their original composition. The amount of fat that was transplanted in this experiment was equivalent to ~10% of total carcass fat as determined by carcass analysis. Although this amount of fat was too small to cause a statistically significant increase in total body fat mass, it was large enough to be detected by the mechanisms that normally control body fat, as evidenced by the reduction in size of the endogenous fat depots of recipient mice 5 wk after the transplants were placed. This suggests that feedback systems controlling

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<th>Sham</th>
<th>Dorsal Recipient</th>
<th>Ventral Recipient</th>
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<td>Presurgical wt, g</td>
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<tr>
<td>Final body wt, g</td>
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<td>Weight change, g/35 day</td>
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</tr>
<tr>
<td>Serum leptin, ng/ml</td>
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<td>0.9±0.2</td>
<td>0.9±0.3</td>
</tr>
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<td>Carcass wt, g</td>
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<td>25.4±0.5</td>
</tr>
</tbody>
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Data are means ± SE for groups of 10 mice.Recipient mice received dorsal or ventral fat transplants of epididymal fat from donor mice 5 wk before determination of carcass analysis.
total fat mass are extremely sensitive and precise and are activated in response to relatively small changes in body fat. The reduction in endogenous fat of recipient mice could have been achieved by either reducing food intake or by increasing expenditure, compared with sham-operated mice. We did not find any significant differences in the food intakes of the groups of mice in experiment 1 and did not measure energy expenditure, although it is unlikely that we would have been able to detect the small change in energy balance that would have been required to account for 500 mg (~4 kcal) of fat over a period of 5 wk.

In experiment 1, there was no obvious change in the size of endogenous fat depots 2 wk after transplant, but there was a compensatory reduction at 5 wk. It is not possible to determine from this study whether the delayed response was due to corrective mechanisms working slowly or whether it took several weeks before the regulatory mechanisms became “aware” of the transplanted fat. We did not determine the vascularization or innervation of the transplanted fat in this study, but others have shown the presence of both blood vessels and nerves in transplanted fat 13 wk after surgery (9). The results of experiment 2, although not statistically significant, tended to support the outcome of experiment 1. The reason for lack of significance in experiment 2 may be associated with the animals in this study being smaller and leaner than those in experiment 1, although the two groups of animals were the same age and strain and were fed the same diet and housed in the same conditions for the two studies. The objective of experiment 2 was to determine whether the location of the fat transplants influenced the response of the animals. The size of endogenous fat depots tended to decrease in both groups of transplant mice, compared with sham-operated animals, so there was no difference between the two groups. The regulation of total body fat has been the subject of investigation for many years but the regulatory mechanisms have yet to be elucidated. Parabiosis studies have illustrated the importance of circulating factors (16) and identification of leptin as the mutated protein in genetically obese ob/ob mice (28) led to the conclusion that leptin plays a critical role in the feedback regulation of body fat mass (5, 10). Contrary to expectations, we recently clearly demonstrated that leptin is not required for mice to compensate accurately for a reduction in body fat mass caused by lipectomy (11), because ob/ob mice, which are leptin deficient, accurately compensated for the removal of body fat by lipectomy. In addition, if leptin acted as a feedback signal to initiate the correction of an increase in total body fat, it would be expected that circulating concentrations of leptin would be elevated before the compensation occurred. In experiment 1, we did not find any elevation of leptin in recipient mice 2 wk after surgery, and leptin was significantly decreased.
5 wk after surgery, when the endogenous fat depots had decreased in size. Because of the results of the parabiosis studies and the increasing number of bioactive proteins that are known to be secreted by adipose tissue, a lot of emphasis has been placed on regulation of total body fat by chemosensitive mechanisms. It is impossible, however, to exclude a role for sensory nerves emanating from white fat (7). The function of these nerves has received little attention, but it is possible that each fat pad provides direct information to the central nervous system on the size of its lipid reserves and that this initiates an effenter neural or hormonal response to compensate for disruptions in total body fat.

In the first experiment, the compensation for the transplanted fat appeared to be achieved primarily through a reduction in the size of existing fat cells. It should be noted that we only measured the size of cells in the epididymal fat depots and it is possible that other fat depots responded by inhibiting the proliferation and/or filling of preadipocytes. It has been reported that when fat depots enlarge, epididymal fat tends to enlarge existing cells, whereas retroperitoneal fat increases cell number (6). Thus it is possible that when these different fat depots reduce in size, the epididymal pad relies on a change in cell size, whereas retroperitoneal reduces the number of new cells that are recruited. The site-specific responses to changes in total body fat are more complex than a simple reliance on a change in either cell number or cell size and may be determined by differences in neural activity (27), hormone responsiveness (8), or differential rates of production of autocrine and paracrine factors (25).

In both experiments described here, the adipocytes in the transplanted fat were smaller than those in the endogenous epididymal fat pads of recipient mice. This reduction in size and decrease in number of measurable cells may have been caused by limited access to nutrients and hormones during the days immediately after surgery, when there would have been a minimal blood supply to the cells. The transplanted cells did not change in size or number between 2 and 5 wk after surgery, which suggests that the systems required to sustain the cells were established within the first 2 wk after the fat was transplanted. As noted above, we did not make any effort to test for vascularization or innervation of the transplanted fat. Casual observation of the transplants indicated that blood vessels had formed around the transplants but the functionality of this fat needs to be determined, as it is possible that the cells had lost some of their metabolic and endocrine function. Clearly, there must have been some blood supply to the transplanted cells; otherwise they would not have survived for 5 wk after the surgery. Serum leptin concentrations were significantly lower in the recipient mice than sham-operated mice in experiment 1, which suggests that the transplanted fat was not secreting leptin to compensate for the reduction in leptin production associated with a reduction in the size of endogenous fat depots.

Lacy and Bartness (20) recently reported that autologous transplants of epididymal fat, but not inguinal fat, in Siberian hamsters stimulate the growth of the nonmanipulated pads over a period of 13 wk. When epididymal fat was added, rather than relocated within the animal, in a design similar to one used here for mice, there was no compensation for the increase in total body fat. There are a number of potential explanations for the differences in response between this study and the study of Lacy and Bartness (20). It is possible that Siberian hamsters, which can change their body fat mass in response to changes in day length, use different mechanism from mice to regulate their body fat, although this does not appear to be likely because the two species show very similar compensatory responses to lipectomy (11, 21). The Siberian hamsters were housed in long days, which induces an increase in fat mass and also is a condition that promotes reproduction. It is possible that the hamsters did not correct for the increase in body fat caused by transplants because they were prepared to respond to the potentially energy expensive conditions of pregnancy and lactation. The paper by Lacy and Bartness (20) includes experiments in which fat was transplanted within an animal. When epididymal fat was placed subcutaneously the hamsters showed an exaggerated enlargement of the nonmanipulated retroperitoneal and inguinal fat depots. This suggests that paracrine factors may play a role in regulating the size of fat depots. These factors do not appear to come into play if the transplanted fat is from a donor animal because the study by Lacy and Bartness (20) showed no change in the endogenous fat depots of recipient hamsters and in experiment 2 we found, if anything, a tendency for dorsal fat transplants to reduce the size of endogenous fat pads.

In summary, the results of this study demonstrate that mice that experience an increase in total body fat due to transplant of exogenous fat respond by reducing the size of their endogenous fat depots. This is one of the first times that the response to a specific increase in body fat mass, caused by a means other than inducing obesity, has been tested. The regulatory system is sensitive to changes in body fat mass that are in the range of 10–15% total body fat, which are too small to cause a statistically significant increase in carcass fat content. The attenuation of endogenous fat is achieved in a depot-specific manner, which includes a reduction in fat cell size, but not cell number, in epididymal fat depots. The mechanisms responsible for the correction of total body fat mass remain to be elucidated but may involve both circulating and neural aspects. Further studies are needed to determine whether these mechanisms are functional in conditions, such as high-fat feeding, that challenge the normal regulation of adiposity.

GRANTS

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