Effect of Leptin Replacement on Intrahepatic and Intramyocellular Lipid Content in Patients With Generalized Lipodystrophy

VINAYA SIMHA, MD[1,3]
LIDIA S. SZCZEPANIAK, PHD[2]
ANTHONY J. WAGNER, PHD[4]
ALEX M. DEPAOLI, MD[4]
ABHIMANYU GARG, MD[1,3]

OBJECTIVE — To investigate whether leptin-induced improvements in glycemic control, hyperlipidemia, and insulin sensitivity in hypoleptinemic patients with generalized lipodystrophies are accompanied by reduction in intrahepatic and intramyocellular lipid (IMCL) accumulation.

RESEARCH DESIGN AND METHODS — We examined the effects 8–10 months of subcutaneous leptin replacement therapy on insulin sensitivity, IMCL, and intrahepatic lipid content in two patients with acquired generalized lipodystrophy and one patient with congenital generalized lipodystrophy. All patients had extreme lack of body fat, low plasma leptin levels, and elevated serum triglycerides, but only two had diabetes. Insulin sensitivity was measured by a high-dose (0.2 IU/kg) insulin tolerance test, as well as by hyperinsulinemic-euglycemic glucose clamp studies in two patients. IMCL and intrahepatic lipid content were measured by ^{1}H magnetic resonance spectroscopy. All measurements were obtained before and during 2–10 months of leptin therapy.

RESULTS — Glycemic control and lipoprotein levels markedly improved with leptin therapy in the two diabetic patients, and a slight improvement in lipoprotein levels was seen in the nondiabetic patients. Insulin stimulated glucose uptake during 60–120 min of the euglycemic clamp studies, and the rate of glucose disappearance during the insulin tolerance test nearly doubled with leptin therapy. As compared with the baseline period, after 8–10 months of leptin therapy, the mean intrahepatic lipid content was reduced by ~80% and the IMCL content was reduced by ~42%.

CONCLUSIONS — Reduction in IMCL and intrahepatic lipid content may partly explain leptin-induced improvement in insulin sensitivity in patients with generalized lipodystrophy.

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Patients with generalized lipodystrophies are characterized by a near-complete absence of adipose tissue and severe insulin resistance and its complications, such as impaired glucose tolerance, early-onset diabetes, acanthosis nigricans, hypertriglyceridemia, low HDL cholesterol concentrations, and fatty liver (1). These patients can have either congenital generalized lipodystrophy (CGL) (also known as Berardinelli-Seip syndrome), an autosomal recessive disorder, or acquired generalized lipodystrophy (AGL), likely an autoimmune disorder. Patients with CGL or AGL have low circulating levels of the adipocyte-derived hormone leptin (2,3). Similar metabolic abnormalities and hypoleptinemia have also been observed in genetically altered mice with lipodystrophies (4,5). In the mouse models, metabolic abnormalities mitigated with adequate amounts of fat transplantation (6) or with leptin administration (5). Recently, in a two-center collaborative study (3), subcutaneous (SC) administration of recombinant human leptin for 4 months was reported to significantly improve glycemic control, insulin resistance, and hypertriglyceridemia in nine patients with lipodystrophies (eight of whom had generalized lipodystrophy). Leptin therapy caused an average 3.6-kg weight loss, and self-reported daily energy intake, measured in seven patients, decreased by an average of 1,080 kcal/day. Interestingly, the insulin tolerance test showed near doubling of the *K* value (rate of glucose disappearance), indicating an improvement in whole-body insulin sensitivity. However, as in the mouse models of lipodystrophy (5,7), energy restriction alone could not fully account for metabolic changes observed with leptin (3).

Recently, there has been accumulating evidence, in both normal lean and obese individuals and in patients with type 2 diabetes, that insulin resistance is strongly associated with increased intramyocellular lipid (IMCL) content (8–12) and with hepatic steatosis (13–15). Interestingly, we previously reported a twofold increase in IMCL content in four patients with CGL (16). Hepatic steatosis has also been reported during early childhood in patients with CGL and AGL (17,18). Therefore, in the present study,

From the [1]Division of Nutrition and Metabolic Diseases, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas; the [2]Division of Hypertension, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas; the [3]Center for Human Nutrition, Unviersity of Texas Southwestern Medical Center, Dallas, Texas; and [4]Amgen, Inc., Thousand Oaks, California.

Address correspondence and reprint requests to Abhimanyu Garg, MD, Chief, Division of Nutrition and Metabolic Diseases, UT Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd., Y3-222, Dallas, TX 75390-9052. E-mail: abhimanyu.garg@utsouthwestern.edu.

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Abbreviations: AGL, acquired generalized lipodystrophy; CGL, congenital generalized lipodystrophy; IMCL, intramyocellular lipid; SC, subcutaneous.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

we investigated three patients, of whom two were previously reported in the above-mentioned study (3), to determine whether leptin-induced improvement in metabolic parameters and insulin sensitivity in patients with generalized lipodystrophy could be partly explained by reductions in IMCL and intrahepatic lipid content.

RESEARCH DESIGN AND METHODS

Patients

Three patients, one with CGL and two with AGL, participated in the leptin trial at the University of Texas Southwestern Medical Center. Informed written consent was obtained, and the study protocol was approved by the Institutional Review Board. The clinical characteristics of the patients studied are briefly summarized below.

Patient 1 was a 31-year-old African-American woman with CGL who has been reported previously (19,20). Recently, compound heterozygous mutations in *AGPAT2* gene were reported in this patient (21). One of her sisters also has CGL. She had extreme lack of body fat and a muscular appearance at birth. Diabetes was diagnosed at age 15 years, and extreme hypertriglyceridemia with tuberoerruptive xanthomas was also noted at the same time. She was being treated with 700 units of insulin a day and fenofibrate, in addition to levothyroxine replacement for postsurgical hypothyroidism. She was also on captopril and hydrochlorthiazide for hypertension. Physical examination revealed a height of 1.67 m and weight of 67.5 kg and was remarkable for a generalized loss of fat from the face, trunk, and extremities; coarse, acromegaloid features; marked acanthosis nigricans over the neck, axillae, and abdominal wall; umbilical hernia; and hepatosplenomegaly. At the time of enrollment in the trial, pooled serum leptin level was low at 0.73 ng/ml.

Patient 2 was a 33-year-old non-Hispanic white woman with AGL. She had juvenile dermatomyositis at age 8 years and received treatment with systemic glucocorticoids, azathioprine, and cyclophosphomide, in addition to plasmapheresis. She has been asymptomatic for the last 10 years, requiring no therapy for dermatomyositis. Fat loss was first noticed from the extremities by age 12 years, and it soon spread to involve the face and trunk. She did not have diabetes but has had hypertriglyceridemia for >15 years, which is being treated with gemfibrozil. She also had primary hypothyroidism requiring levothyroxine replacement. Physical examination revealed a height of 1.62 m, weight of 47.6 kg, and marked loss of SC adipose tissue from the face and extremities, including the palms and soles. SC fat was also decreased on the trunk except for the anterior abdomen, where it was still preserved. She had acanthosis nigricans over the axillae and neck and had mild hepatomegaly. A few irregular, hard calcified deposits were also palpable in the muscles of her arm, forearm, and abdomen. Her pooled serum leptin concentration was 2.35 ng/ml.

Patient 3 was a 15-year-old white boy from Kazakhstan who had AGL. Loss of SC fat was first noticed around the age of 5 years, whereas diabetes and hypertriglyceridemia were detected at age 10 years. Both these conditions were managed by diet therapy alone. He also had a congenital bicuspid aortic valve but was asymptomatic. Physical examination revealed a height of 1.82 m, weight of 39.6 kg, and marked loss of SC adipose tissue from the face, trunk, and extremities, including the palms and soles. There was no acanthosis nigricans. The liver was palpable 5 cm below the right costal margin. His pooled serum leptin level was 0.07 ng/ml.

Study design

The details of the study design have been published earlier (3). Patients were admitted to the General Clinical Research Center at University of Texas Southwestern Medical Center, Dallas, for initial evaluation before recombinant human leptin treatment and subsequently after 2, 4, and 8 months of recombinant human leptin therapy. However, patient 3 was evaluated at 3, 6, and 10 months after the baseline evaluation. Except for hypoglycemic drugs, which were tapered or discontinued as needed, none of their other concomitant medications were changed during the study period.

Human recombinant leptin was provided by Amgen, Inc. (Thousand Oaks, CA). It was administered subcutaneously every 12 h to achieve near-physiological concentrations of plasma leptin. The physiological replacement dose was estimated to be 0.04 mg $\cdot$ kg^{-1} $\cdot$ day^{-1} for women and 0.03 mg $\cdot$ kg^{-1} $\cdot$ day^{-1} for men. Patients were treated with 50% of the replacement dose for the first month, 100% during the second month, and 200% subsequently.

Measurement of insulin sensitivity

A high-dose insulin tolerance test using 0.2 IU/kg regular insulin (Novolin R; Novo Nordisk, Princeton, NJ) was performed on all the patients to assess insulin sensitivity at baseline and periodically during 2–10 months of leptin therapy as described earlier (3). Further, in patients 2 and 3, we performed euglycemic-hyperinsulinemic glucose clamp studies at baseline and after leptin therapy (4 months of leptin therapy in patient 2 and 10 months in patient 3) using an insulin infusion rate of 80 mU $\cdot$ m^{-2} $\cdot$ min^{-1} for 120 min according to the protocol described earlier (22).

^{1}H magnetic resonance spectroscopy

We performed ^{1}H magnetic resonance spectroscopy studies during the baseline period and at 2, 4, and 8 months after starting leptin therapy in patients 1 and 2, at baseline, and at 6 and 10 months after leptin therapy in patient 3. All measurements were performed at least 4 h postprandially with the patients lying supine. The details of the technique used have been described earlier (16). Briefly, image-guided proton-localized ^{1}H magnetic resonance spectroscopy and high-resolution T1-weighted imaging were performed on a 1.5 T Gyroscan NT whole body system (Philips Medical Systems, Best, the Netherlands) with the following imaging parameters: repetition time of 500 ms, echo time of 33 ms for the muscle and 40 ms for the liver, and 1,024 data points over a 1,000-kHz spectral width. Volumes of interest (voxel) within muscle were centered over the mid-soleus (12–15 mm [3]) and on the right lobe of the liver (30–50 mm [3]), taking care to avoid vascular structures and gross adipose tissue deposits and to ensure consistent orientation of muscle fibers along the magnetic field. Spectra were processed and resonances quantified using a standard analysis package (NUTS; ACORNNMR, Fremont, CA). The intramyocellular and intrahepatic lipid concentration is expressed as a percentage of the intensity of the water resonance peak.

RESULTS — The baseline characteristics of patients 1 and 2 and the effect of 4 months of leptin replacement on their physical and metabolic parameters have been described previously (3). Briefly, in patient 1, leptin replacement resulted in a weight loss of 3 kg, a decrease in HbA_{1c} concentration from 9.5 to 7.3%, and a reduction in fasting serum triglycerides from 11.23 to 2.09 mmol/l. Her serum leptin levels increased from 0.73 to 3.3 ng/ml. Similarly in patient 2, the body weight decreased from 47.6 kg at baseline to 45.6 kg at 4 months. Her HbA_{1c} concentration did not change, whereas fasting serum triglycerides decreased slightly from 5.05 mmol/l at baseline to 4.79 mmol/l at 4 months. Serum leptin levels increased from 2.35 to 8.5 ng/ml. Continued leptin therapy has caused further improvements in the metabolic parameters in both the patients at the end of 8 months. Similarly, 10 months of leptin therapy in patient 3 resulted in normalization of fasting plasma glucose from 11.6 to 4.5 mmol/l. Likewise, his HbA_{1c} declined from 10.7 to 6.7%, and fasting serum triglycerides declined from 9.14 to 1.74 mmol/l. During this period, his body weight increased slightly from 39.6 to 40.2 kg.

Leptin replacement led to improvement in whole-body insulin sensitivity in all three patients. After 8–10 months of leptin therapy, the rate of glucose disappearance during the insulin tolerance test, expressed as the *K* value, increased from 0.44 to 3.1% in patient 1, from 1.58 to 1.67% in patient 2, and from 0.66 to 1.36% in patient 3 (Fig. 1*C*). Hyperinsulinemic-euglycemic glucose clamp studies on patient 2 revealed an increase in the mean 60- to 120-min insulin-stimulated glucose uptake (*M* value) from 3.41 mg · kg^{-1} · min^{-1} at baseline to 3.73 mg · kg^{-1} · min^{-1} after 4 months of leptin therapy. Similar mean serum insulin levels were achieved during the hyperinsulinemic phase of the clamp studies (548 pmol/l during the baseline period and 616 pmol/l at 4 months). Mean fasting serum insulin level reduced from 78 to 41 pmol/l after 4 months of leptin therapy. In patient 3, the *M* value increased from 0.45 mg · kg^{-1} · min^{-1} at baseline to 3.72 mg · kg^{-1} · min^{-1} after 10 months of leptin therapy. The mean serum insulin levels achieved during the hyperinsulinemic phase of the clamp studies were 1,008 pmol/l during the baseline period and 1,151 pmol/l at 10 months. The fasting serum insulin level had decreased from 316 pmol/l at baseline to 119 pmol/l at 10 months.

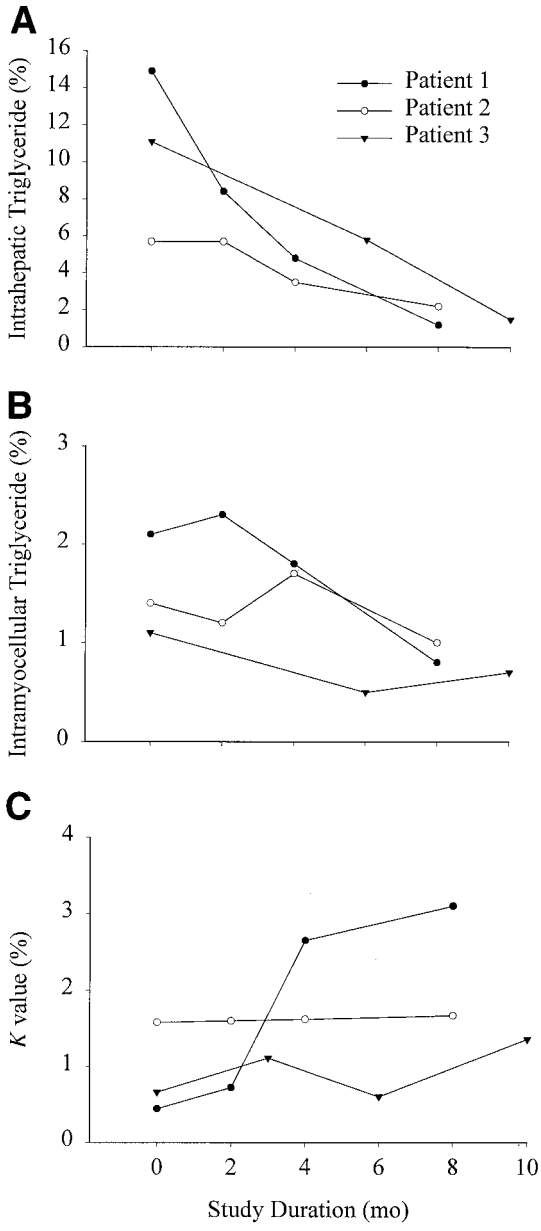

Figure 1—*Changes in intrahepatic* (A) *and intramyocellular* (B) *lipid content in three patients with generalized lipodystrophy treated with human recombinant leptin for 8–10 months. The corresponding changes in insulin sensitivity, measured as the rate of glucose disappearance during the insulin tolerance test* (K *value) are shown in* C.

Figure 1*A* shows the changes in intrahepatic triglyceride levels with leptin therapy. During the baseline period, the intrahepatic lipid content in patients 1 and 3 were about two- to threefold higher than normal, whereas in patient 2, it was in the high-normal range. During leptin therapy, the hepatic fat content progressively decreased from 14.9% at baseline to 1.2% at the end of 8 months in patient 1, from 5.7 to 2.2% in patient 2, and from 11.1 to 1.5% in patient 3.

Figure 2 shows a transaxial T1-weighted magnetic resonance image of the calf in patients 1 and 2, indicating the position of the voxels. Striking absence of SC adipose tissue can be noted in both the patients. Lack of bone marrow fat and intermuscular fat is apparent in patient 1 with CGL, whereas these compartments are well preserved in patient 2. Changes in IMCL content with leptin therapy are shown in Fig. 1*B*. In patient 1, IMCL decreased from 2.1% at baseline to 0.8% at the end of 8 months; in patient 2, it declined from 1.4 to 1.0%, and in patient 3 from 1.1 to 0.7%.

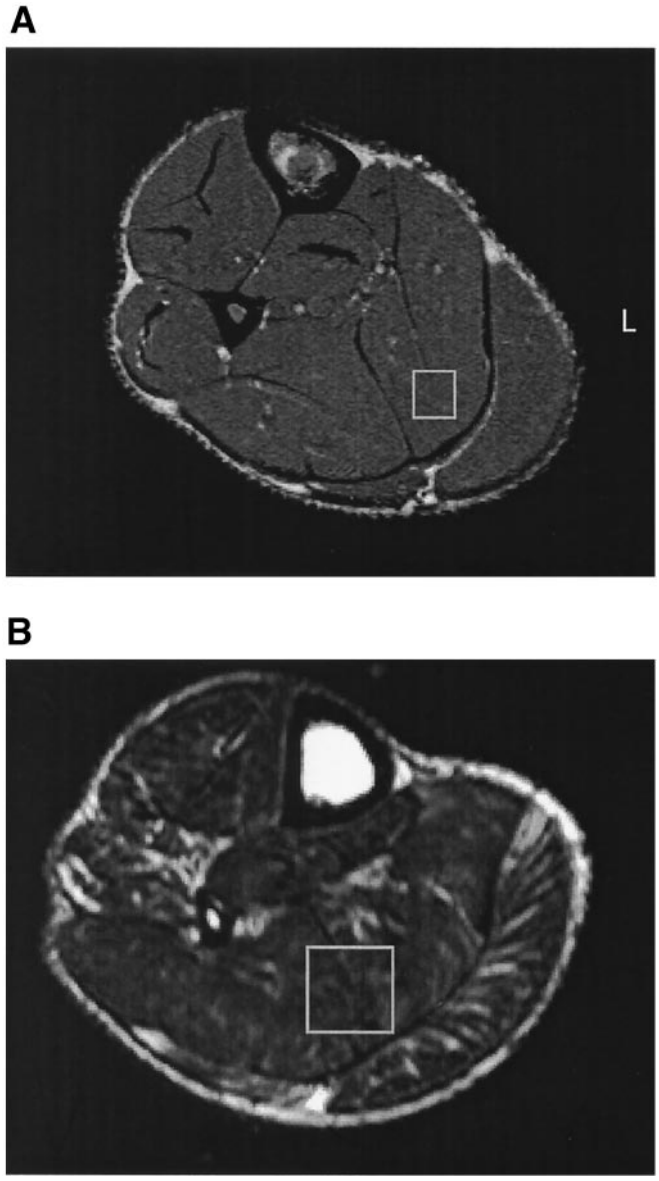

Figure 2— *T1-weighted transaxial magnetic resonance image of the calf in patient 1 (*A*) and patient 2 (*B*) showing position of the voxels over the soleus muscle. SC adipose tissue is markedly reduced in both the patients, whereas bone marrow fat and fat in the intermuscular fasciae are absent only in patient 1, who has CGL. L, left side.*

CONCLUSIONS — Leptin replacement in lipodystrophic patients significantly improved the metabolic abnormalities associated with insulin resistance (3). In the current study, we have noticed that this improvement is associated with a marked reduction in intrahepatic lipid content and a modest decline in IMCL content. The 8–10 months of treatment with human recombinant leptin at a near-physiological replacement dose resulted in a mean decline in intrahepatic lipid content of ~80%. Furthermore, as reported previously, 4-month-long leptin therapy had caused an average 28% reduction in liver volume in nine patients with lipodystrophy (3). The magnitude of changes noticed in these three case reports suggests that leptin almost reverses hepatic steatosis in generalized lipodystrophy. Similarly, we noted a mean reduction in IMCL content of ~42% in our study. It appears that one of the important actions of leptin is to reduce the intracellular triglyceride content in non–adipose tissue.

Experience with leptin therapy in humans is limited. Leptin has previously been administered to a 9-year-old girl with congenital leptin deficiency (23) and to normal lean and obese adults for weight loss (24). Leptin therapy for 12 months in the girl with congenital leptin deficiency caused a weight loss of 16.4 kg, with fat loss accounting for 95% of it. There were, however, no changes in fasting serum glucose, insulin, or triglyceride levels with leptin therapy (23). Similarly, Heymsfield et al. (24) reported a dose-dependent weight loss (predominantly fat loss) ranging from 0.4 to 1.9 kg with escalating doses of leptin in 54 lean and 73 obese subjects. The investigators reported no changes in serum glucose or insulin profiles during the oral glucose tolerance test with leptin therapy. Both of these studies, however, did not examine intrahepatic or IMCL content.

Leptin has been found to be efficacious in reducing intrahepatic lipid concentration in mouse models of lipodystrophy as well as obesity. Marked hepatic steatosis in lipodystrophic mice can be reversed by leptin administration (5,7) or by transgenic overexpression of leptin (7). Furthermore, liver-specific overexpression of wild-type leptin receptors in Zucker diabetic fatty (ZDF *fa/fa*) rats (which have a loss of function mutation in leptin receptors) reduced excessive triglyceride accumulation in the liver (25). There are no data, however, on effects of leptin on IMCL content in mouse models.

The mechanism of leptin's seemingly "liporegulatory" role is not known, but leptin presumably directly affects either lipid oxidation or synthesis. Using microarray expression profiles, Ferrante et al. (26) showed that leptin altered expression of a wide variety of genes involved in fatty acid metabolism in the livers of leptin-deficient *ob/ob* mice, including reduced suppression of insulin-induced protein 2, which may have a role in fatty acid overproduction. However, Lee et al. (25) suggest that the anti-steatotic action of leptin in the liver is due to enhanced fatty acid oxidation. They noted increased expression of peroxisome proliferator–activated receptor-α and carnitine-palmitoyl transferase 1 in the livers of normal rats on a high-fat diet, which they ascribed to the effect of hyperleptinemia. Based on these observations, they conclude that the physiological role of hyperleptinemia secondary to energy excess is to protect non–adipocytes from steatosis and lipotoxicity.

As previously mentioned, various cross-sectional studies have identified IMCL content as an important determinant of whole-body insulin sensitivity (9–12). Furthermore, IMCL concentrations decrease with exercise and weight loss, interventions that also improve insulin sensitivity (27–29). However, recent reports of increased IMCL content in endurance-trained athletes who are extremely insulin sensitive present a paradox (30). It has been suggested that the capacity for lipid oxidation may be a more important mediator of the association between excess muscle lipid accumulation and insulin resistance. Interestingly, leptin stimulates fatty acid oxidation in skeletal muscle by activating AMP-activated protein kinase and inhibiting acetyl CoA carboxylase (31). It is therefore conceivable that a leptin-induced increase in fatty acid oxidation caused a reduction in IMCL accumulation and an improvement in peripheral insulin sensitivity in our patients with lipodystrophy. Even though we noticed an overall reduction in IMCL content with leptin therapy, there were small transient increases in IMCL during the study, whereas reductions in intrahepatic lipid content were more pronounced, consistent, and progressive with the duration of leptin therapy. It is not clear whether these differences are related to methodological difficulties associated with separation of intra- and extramyocellular lipid spectra or to the dynamics of lipid oxidation and storage with changing insulin sensitivity.

We conclude that leptin replacement in hypoleptinemic patients with generalized lipodystrophy decreased intrahepatic and IMCL content, which may partly explain improvement in insulin sensitivity with leptin therapy.

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ADDENDUM— Since the submission of this manuscript, similar results have been reported by Petersen et al. (*J Clin Invest* 109:1345–1350, 2002) in three women with generalized lipodystrophy. The authors reported an 86% reduction in hepatic triglyceride content and a 33% reduction in muscle triglyceride content after 3–8 months of leptin therapy using the same treatment protocol as ours.

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