Bromocriptine/SKF38393 Treatment Ameliorates Dyslipidemia in ob/ob Mice

Ying Zhang, Piotr W.D. Scislowski, Robert Prevelige, Sharon Phaneuf, and Anthony H. Cincotta

Our previous studies have shown that the dopaminergic D_1 receptor agonist SKF38393 (SKF) plus the D_2 receptor agonist bromocriptine (BC) act synergistically to reduce obesity in obese C57BL/6J (ob/ob) mice. The present study investigated the effects of this combination on dyslipidemia in ob/ob mice. Female ob/ob mice were treated daily with vehicle or SKF (20 mg/kg body weight [BW]) plus BC (16 mg/kg BW [BC/SKF]) for 14 days. The animals were then used for the characterization of plasma lipoprotein profiles, hepatic triacylglycerol synthesis and secretion, adipocyte lipolysis, adipose and muscle lipoprotein lipase (LPL) activity, and muscle triglyceride (TG) content. The treatment significantly reduced plasma glucose 54%, TG 41%, cholesterol 21%, phospholipid 20%, and free fatty acid (FFA) 36% ($P < .01$). Hepatic triacylglycerol synthesis was 55% lower in treated mice versus control mice ($P < .01$). The cell size of isolated adipocytes was significantly reduced (41%) by treatment. LPL activity was increased in soleus skeletal muscle (25%, $P < .05$) but was sharply reduced in adipose tissue (91%, $P < .01$) in treated versus control mice. The TG content of hindlimb muscle was about 49% lower in treated versus control mice ($P < .05$). The basal and isoproterenol-stimulated lipolytic rate was decreased (approximately 53%) in adipocytes from treated animals compared with the control ($P < .01$). In conclusion, BC/SKF normalized the hypertriglyceridemia likely via its simultaneous antilipogenic action in liver tissue and antilipolytic action in adipose tissue. Decreased plasma flux of FFA partially contributed to the reduced hepatic lipogenesis, plasma very-low-density lipoprotein (VLDL)-TG, and TG in skeletal muscle. The above-described effects of BC/SKF treatment are largely independent of its effect to normalize hyperphagia in ob/ob mice.

DYSLIPIDEMIA in the form of elevated triglyceride (TG)-rich lipoprotein and serum free fatty acid (FFA) levels is a common abnormality associated with type 2 diabetes.[1-3] Increasing evidence indicates that elevated serum TG is a risk factor for coronary heart disease[4-6] and that high serum FFA and TG levels can potentiate insulin resistance.[7-10] Despite progress in delineating the etiologic role of dyslipidemia in the pathophysiology of diabetes, we are still searching for insights into the physiologic regulatory alterations responsible for the initial development of dyslipidemia.

Observations by several laboratories have indicated that increased TG synthesis, secretion, storage, and subsequent mobilization (ie, increasing serum FFA) is a naturally occurring seasonal phenomenon in vertebrates.[11] Vertebrates synthesize and then subsequently utilize large amounts of lipid at particular times of the year, enabling them to survive long periods of low food availability.[11] Our laboratory has demonstrated that natural seasonal or pharmacological increases in dopaminergic tone shift lipid metabolism from high to low lipid synthesis and mobilization.[11-14] Recently, we have applied this "natural" approach of regulating metabolism to the treatment of lipid (and carbohydrate) abnormalities in the genetically obese, hyperglycemic C57BL/6J mouse (ob/ob). These mice have increased rates of TG synthesis and secretion, as well as mobilization, both contributing to elevated serum lipids.[15-17] Compared with lean mice, ob/ob mice have plasma TG levels that are elevated as much as 1.5-fold to twofold[18] and characterized as hypertriglyceridemic, reported to be 199 ± 25 mg/dL in the fasting state.[19] These animals also have an elevated TG content in skeletal muscle,[20] which may potentiate insulin resistance.[21,22] Treatment of ob/ob mice with the dopamine agonists bromocriptine ([BC] D_2 receptor agonist) plus SKF38393 ([SKF]D_1 receptor agonist) has been shown to reduce obesity, hyperglycemia, and hyperlipidemia independent of its effects to reduce feeding.[23]

This study investigates the metabolic activities involved in the amelioration of dyslipidemia in these mice by such dopamine agonist treatment. Specifically, we examined the in vivo dopamine agonist treatment effects on (1) liver TG esterification, secretion, and subsequent removal in adipose tissue and muscle (ie, lipoprotein lipase [LPL] activities), (2) basal and isoproterenol-stimulated adipocyte lipolysis, (3) skeletal muscle TG content, and (4) serum lipoprotein profile.

MATERIALS AND METHODS

Experimental Design

Six-week-old obese female C57BL/6J mice (ob/ob) (body weight [BW], 44.5 ± 0.5 g) were supplied by Jackson Laboratory (Bar Harbor, ME) and maintained in a temperature-controlled (18° ± 2°C) room with a 12-hour light/dark cycle. Animals were divided into three groups: vehicle control, pair-fed control, and treated. They were singly housed and allowed to adapt to the climate-controlled environment for at least 1 week before studies began. In our experience, singly housing ob/ob mice produces a consistent and uniform hyperglycemia among animals. Free access to standard chow diet (Prolab RMH 3000; Agway, Syracuse, NY) and water was allowed throughout the experiment, except in the pair-fed group. Animals were injected intraperitoneally daily at 1 hour after light onset (HALO) for 14 days with vehicle or SKF (20 mg/kg BW) plus BC (16 mg/kg BW [BC/SKF]) simultaneously. Food consumption was measured daily, and BW was monitored on days 0, 7, and 14 of treatment. The amount of food given to pair-fed animals was calculated from the consumption on the prior day by treated animals, and was offered to the animals at 11 HALO to coincide with the natural onset of feeding in these nocturnal animals. On day 15 of the study, serum was isolated at 1 HALO (24 hours following the final treatment) for analysis of insulin, glucose, lipids, and the chemical composition of serum lipoproteins. We also investigated the effect of treatment on the incorporation of ^{3}H-glycerol into hepatic TG and on the hepatic TG secretion rate. We also examined LPL activity in heart, skeletal muscle, and parametrial adipose tissue. Adipose tissue was also taken for the determination of cell size and lipolytic rate. The TG content of heart and skeletal muscle was measured. Inasmuch as

From ErgoScience Corp, Charlestown, MA.
Submitted October 7, 1998; accepted February 19, 1999.
Address reprint requests to Anthony H. Cincotta, PhD, ErgoScience Corp, 100 First Ave, Charlestown, MA 02129.

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BC/SKF treatment normalizes hyperphagia, some studies included a diet-restricted control (pair-fed to BC/SKF-treated mice).

Chemicals

Solvents and chemicals were the highest grade commercially available from Fisher Chemical (Fair Lawn, NJ). Superose CL 6B, Triton WR-1339, KBr, triolein, and egg yolk phosphatidylcholine were obtained from Sigma (St. Louis, MO). Tri-[9,10-^{3}H]oleate was purchased from ICN (Costa Mesa, CA). ^{3}H-glycerol was obtained from New England Nuclear (Boston, MA).

Plasma Metabolite Analyses

Plasma glucose was assayed with a glucose monitor (Accu-Check Advantage; Boehringer, Indianapolis, IN). Plasma cholesterol and TG concentrations were determined by commercially available colorimetric enzymatic assay kits (Sigma). Plasma FFA, free cholesterol, and phospholipid levels were determined by enzymatic assay with kits purchased from Wako (Richmond, VA). The plasma insulin concentration was measured by radioimmunoassay using rat insulin as a standard and anti–rat insulin antibody with kits from Linco Research (St. Charles, MO).

Measurement of ^{3}H-Glycerol Incorporation Into Liver TG

Animals were injected intraperitoneally with 2-^{3}H-glycerol (20 μCi per mouse, 17.4 Ci/mmol) in 100 μL 0.15-mol/L NaCl at either 4, 8, 12, 14, or 16 HALO to evaluate TG reesterification at various times in the lipolytic and lipogenic periods of the day.[15] After 30 minutes, the animals were killed and the plasma was prepared. Liver and parametrial adipose tissues were rapidly frozen for determination of ^{3}H-glycerol incorporation into TG. Lipids contained within these tissues were extracted with chloroform-methanol as previously described.[24] With this method, it has been shown that greater than 90% of the radioactivity is present in extracted lipids recovered in the TG fraction.[25] The recovery of TG radioactivity was used to calculate the incorporation of ^{3}H-glycerol into hepatic TG and adipose TG based on the specific radioactivity of plasma ^{3}H-glycerol. The incorporation rate is expressed as micrograms of glycerol incorporated per minute per gram of tissue.

Measurement of Hepatic TG Secretion Rate

To assay hepatic TG secretion, Triton WR-1339 (15% Triton wt/vol) at a dose of 300 mg/kg BW was injected into the tail vein at 1 HALO. Blood samples for measurement of TG were obtained at 0, 30, 60, and 90 minutes after injection of Triton. TG accumulation rates were determined similarly to the method of Kasim et al[26] as follows: rate (milligrams per hour) = ½[(TG30 − TG0)/0.5 + (TG60 − TG0) + (TG90 − TG0)/1.5] × plasma volume, where TG30, TG60, and TG90 are TG concentrations at 30, 60, and 90 minutes after Triton injection, respectively, and TG0 is the TG concentration before injection. The plasma volume used was established for mice at 5.77% of BW.[27]

Isolation and Characterization of Plasma Lipoproteins

A separate group of animals treated as before were used for the analysis of BC/SKF effects on the serum lipoprotein profile. Before separation of lipoproteins, plasma chylomicrons were removed by centrifugation at 15,000 × *g* for 30 minutes at 4°C. Two methods were subsequently used for fractionation of plasma lipoproteins: (1) lipoproteins were separated by a single discontinuous density gradient ultracentrifugation[28] and fractionated into density classes of less than 1.006 (very–low-density lipoprotein [VLDL]), 1.006 to 1.063 (low-density lipoprotein [LDL]), and 1.063 to 1.21 (high-density lipoprotein [HDL]); and (2) molecular filtration on column chromatography was performed with Superose 6B gel with a slight modification for lipoprotein isolation.[29] Plasma (200 μL) was applied to two Superose 6B columns (10 × 300 mm) connected in series. Chromatography was performed at a constant flow rate of 12 mL/h. Eluent fractions of 400 μL each were collected after discarding the first 12 mL. Continuous monitoring of the 280-nm absorbancy of the eluate was performed to detect irregularities and to monitor the complete elution of plasma protein. Aliquots of collected lipoprotein fractions from both methods were used to determine the lipid content by colorimetric enzymatic assay with commercially available kits (Sigma). Protein content was measured according to the method of Lowry et al.[30]

LPL Assay

Heart, parametrial adipose tissue, and soleus muscle pairs were removed from the mice following death. Tissue was cut into small pieces and placed in vials containing 0.4 mL Hanks buffer with sodium heparin (200 μg/mL). Each sample was assayed in duplicate. After a 40-minute incubation in a shaking water bath at 37°C, 0.1 mL incubation medium was removed to a new vial and LPL activity was measured with 0.1 mL substrate emulsion as described by Hietanen and Greenwood,[31] containing 1.5 μmol triolein and 1.6 μCi glycerol tri-[9,10-^{3}H]oleate, 9 μg egg yolk phosphatidylcholine sonicated in 67 μL buffer (0.3 mol/L Tris hydrochloride and 3% BSA, pH 8.6), and 33 μL heat-inactivated human serum (total reaction vol, 0.2 mL). The enzymatically released fatty acid was extracted as described by Belfrage and Vaughan,[32] and radioactivity in the aqueous phase was counted in a liquid scintillation counter. LPL activity was expressed as micromoles of FFA released per hour per gram of tissue. It was also expressed as micromoles of FFA released per hour per 10^6 cells for adipose tissue.

Heart and Skeletal Muscle TG Content

Following death, the heart and total skeletal muscle tissue dissected from the hindlimb were placed in liquid nitrogen. The tissues were extracted in chloroform/methanol,[24] and after separation, the chloroform phase was retained and evaporated to dryness under nitrogen. The residue was reconstituted in 200 μL chloroform. A 2.5-μL aliquot was used to determine TG content with the enzymatic method as already described in a final reaction volume of 1 mL.

Lipolysis in Isolated Adipose Cells

Adipose cells were isolated from parametrial adipose tissue of individual mice by a collagenase digestion method.[33] Digestion was performed with 1 g minced fat pad in 2 mL 1-mg/mL collagenase for 45 minutes in 2.5% Fraction V BSA in Krebs-Ringer phosphate buffer at 37°C in a shaking water bath. Cells were then filtered through nylon mesh. The cell diameter was measured under a microscope,[34] and lipid content was measured by lipid extraction. Cell size was expressed as micrograms of lipid per cell.[35] A 10% suspension of isolated adipocytes (one part cells per nine parts media) from parametrial adipose tissue of individual mice was prepared in Krebs-Ringer media buffered with 20 mmol/L HEPES, pH 7.4, containing 200 nmol/L adenosine, 2 mmol/L glucose, and 2.5% BSA.[36] Cell incubations were initiated by adding 100 μL adipocyte suspension to incubation vials containing 400 μL media with 1 U/mL adenosine deaminase (Sigma) and lipolytic stimulators (isoproterenol). Incubations were performed at 37°C in a shaking water bath for 20 minutes. Lipolysis was determined by vortexing the cells through dinonyl phthalate oil. The glycerol content in 150-μL aliquots of cell-free media was measured using a colorimetric enzymatic assay kit (Sigma).

Statistical Analysis

Data are presented as the mean ± SEM. Statistical significance between groups was calculated by Student's *t* test.

RESULTS

BW, Food Consumption, and Plasma Metabolites

After 2 weeks of treatment, daily food consumption and BW gain were significantly reduced (by 45% and 14%, respectively, $P < .01$) in BC/SKF-treated ob/ob mice compared with controls (Fig 1). Pair-feeding also reduced the BW gain (10%, $P < .01$). BC/SKF also reduced plasma levels of glucose (54%, from 407 ± 18 to 186 ± 27 mg/dL, $P < .01$), insulin (50%, from 48 ± 4 to 24 ± 4 ng/mL, $P < .01$), TG (41%, from 322 ± 22 to 191 ± 8 mg/dL, $P < .01$), FFA (36%, from 1.25 ± 0.04 to 0.8 ± 0.06 mmol/L, $P < .01$), cholesterol (21%, from 168 ± 7 to 132 ± 8 mg/dL, $P < .01$), and phospholipid (20%, from 297 ± 12 to 238 ± 17 mg/dL, $P < .01$) relative to controls. However, plasma glucose (326 ± 37 mg/dL) and FFA (1.3 ± 0.01 mmol/L) were not reduced in the pair-fed group versus the control group. Regarding lipids, pair-feeding reduced plasma total TG 17% (from 322 ± 22 to 267 ± 13 mg/dL, $P < .05$). However, BC/SKF treatment further reduced plasma total TG 28% (from 267 ± 18 to 191 ± 8 mg/dL, $P < .05$) relative to the pair-fed group. Plasma cholesterol and phospholipid levels were similarly reduced by pair-feeding and BC/SKF treatment relative to controls (Table 1).

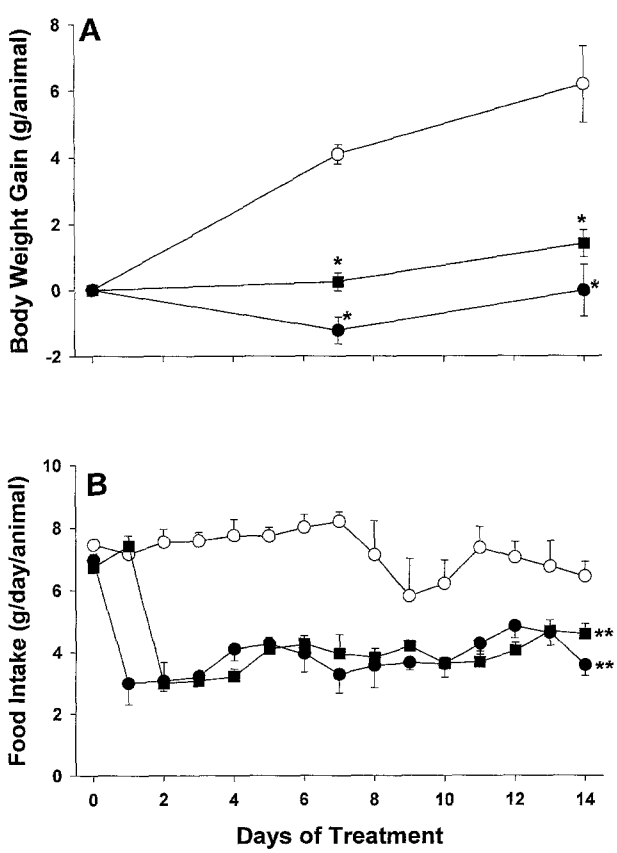

Fig 1. Effects of BC/SKF treatment on BW and daily food intake in ob/ob mice. (A) BW was monitored at 0, 7, and 14 days after treatment and (B) food consumption was measured daily during treatment in control (○), pair-fed (■), and BC/SKF (●) mice. Results are the mean ± SE of 6-8 mice per group. *$P < .01$ *v* controls. **Significant reduction in daily food consumption over the 2-week treatment period, $P < .05$.

Table 1. Effects of BC/SKF Treatment on Blood Lipids

Group	FFA (mmol/L)	TG (mg/dL)	CH (mg/dL)	PHOS (mg/dL)
Control	1.1 ± 0.05	322 ± 22	167 ± 7	297 ± 12
BC/SKF	0.8 ± 0.03*†	191 ± 8*†	132 ± 8*	238 ± 17*
Pair-fed	1.3 ± 0.1*	267 ± 13*	142 ± 7*	244 ± 9*

NOTE. Results are the mean ± SE of 6-8 mice per group.
Abbreviations: CH, cholesterol; PHOS, phospholipids.
*$P < .05$ *v* ob/ob controls.
†$P < .05$ *v* pair-fed controls.

^{3}H-Glycerol Incorporation Into TG in Liver and Adipose Tissue

Many reports have indicated that there is a significant diurnal rhythm in lipid synthesis and mobilization in rodents, including mice.[15] Therefore, the circadian aspects of TG synthesis were investigated. Figure 2 shows the effects of BC/SKF treatment on the daily variation of glycerol incorporation into hepatic TG in ob/ob mice. The maximum rate of synthesis of liver TG occurs in the late part of the dark period to early morning. Following BC/SKF treatment, glycerol incorporation into liver TG was reduced approximately 55% (23 ± 1.7 *v* 11 ± 2.6 μg/min/g, $P < .01$) during this peak synthetic period of the day. Similar results were found in adipose tissue, where the incorporation of glycerol into TG was reduced about 57% during the peak period (3 ± 1.2 *v* 1.3 ± 0.6 μg/min/g, $P < .05$).

Hepatic TG Secretion

To directly test the possibility that the decrease in plasma TG after BC/SKF treatment may be due to a decrease in hepatic secretion of VLDL, we blocked the clearance of TG-rich lipoprotein by intravenous injection of Triton WR-1339. During the next 2 hours, the rate of plasma TG entry was 16.3 ± 2.1 mg/h in BC/SKF-treated ob/ob mice and 27.1 ± 1.9 mg/h in control ob/ob mice ($P < .01$).

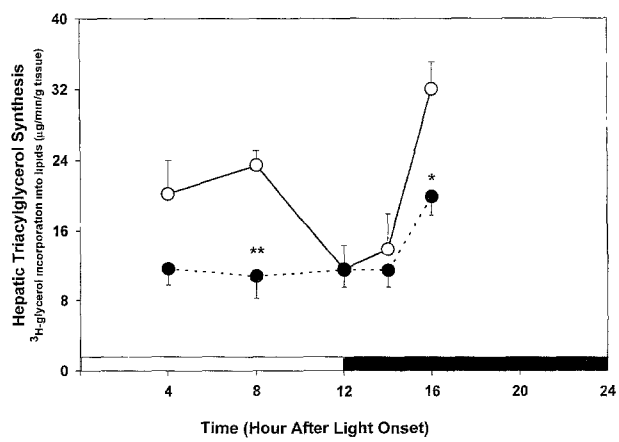

Fig 2. Effects of BC/SKF treatment on ^{3}H-glycerol incorporation into liver lipids in ob/ob mice. Control (○) and BC/SKF (●) mice received ^{3}H-glycerol at 4, 8, 14, and 16 HALO. At 30 minutes after injection, the animals were killed and the livers were rapidly frozen and homogenized in chloroform-methanol (2:1 vol/vol). Thereafter, lipids were extracted, and incorporation of ^{3}H-glycerol into fatty acid was determined. Results are the mean ± SE of 6-8 mice per group. *$P < .05$ and **$P < .01$ *v* controls.

Table 2. Effects of BC/SKF Treatment on Chemical Composition of Serum Lipoproteins

Lipoprotein (mg/dL)	TG	CE	FC	PHOS	PRO
Total serum					
Control	341 ± 20	—	—	380 ± 11	—
BC/SKF	162 ± 10†	—	—	287 ± 15*	—
Non-CHYL					
Control	173 ± 10.1	—	—	—	—
BC/SKF	120 ± 4†	—	—	—	—
VLDL					
Control	108.2 ± 9.6	4.8 ± 0.3	4.7 ± 0.1	23 ± 1.7	18.5 ± 0.7
BC/SKF	44.4 ± 3.9†	3.9 ± 0.5	3.9 ± 0.4	5.5 ± 2.4*	15.1 ± 0.7
LDL					
Control	37.8 ± 3.3	22.8 ± 1.9	7.4 ± 1	57.5 ± 5.1	23.2 ± 4
BC/SKF	20.1 ± 2.5*	22.5 ± 2.1	6.0 ± 0.5	23.0 ± 1.7*	24.3 ± 2
HDL					
Control	2.8 ± 0.2	60 ± 4	11.9 ± 1.5	77.5 ± 6	162.9 ± 15
BC/SKF	1.4 ± 0.1*	45.7 ± 5.2*	20.0 ± 3.9	52.2 ± 4*	151.6 ± 10

NOTE. Results are the mean ± SEM of 6-8 animals per group. Each analysis was made in duplicate.
Abbreviations: CE, cholesterol ester; FC, free cholesterol; PHOS, phospholipids; PRO, protein; Non-CHYL, chylomicron-free serum.
*$P < .05$, †$P < .01$: control *v* SKF/BC-treated ob/ob mice.

Plasma Lipoprotein Profile

The TG and phospholipid content of each lipoprotein fraction (VLDL, LDL, and HDL) isolated from both methods was significantly decreased by 47% to 59% and 33% to 76%, respectively, in treated versus control mice ($P < .05$). The small decrease in cholesterol was mainly due to a decrease in HDL cholesterol, which is the primary cholesterol carrier in mice.[37] The decrease of nonchylomicron plasma TG was predominately due to a decrease in the amount of VLDL-TG (Table 2).

LPL Activity in Heart, Skeletal Muscle, and Adipose Tissue

The effects of BC/SKF on tissue LPL activities are shown in Fig 3. In cardiac muscle, we observed the highest LPL activity of all peripheral tissues examined, which was not significantly altered either by the treatment or by pair-feeding. In adipose tissue, LPL activities were decreased approximately 83% by BC/SKF when expressed as micromoles of FFA released per gram of tissue per hour ($P < .01$). BC/SKF also reduced adipose cell size by 41% from 1.04 ± 0.07 to 0.61 ± 0.05 μg lipid per cell ($P < .001$), so the treatment-induced reduction of LPL activity was even more obvious (91%) when expressed as micromoles of FFA released per 1 million cells per hour ($P < .001$). In contrast, adipose LPL activities were not significantly different in pair-fed mice versus ob/ob controls when expressed either as micromoles of FFA released per gram of tissue per hour (4.3 ± 1.1 *v* 4.6 ± 0.8) or as micromoles of FFA released per 1 million cells per hour (13.4 ± 3.4 *v* 16.7 ± 2.9). Interestingly, there was an increase of LPL in skeletal muscle tissue following BC/SKF treatment (about 64%, $P < .05$), but not following pair-feeding for 2 weeks, compared with controls. Parametrial adipose cell size was not significantly changed in pair-fed mice compared with controls (1.04 ± 0.07 to 0.89 ± 0.04 μg lipid per cell).

TG Content of Heart and Skeletal Muscle Tissue

The TG content of skeletal muscle was about 51% lower in BC/SKF-treated versus control mice (3.1 ± 0.5 *v* 6.1 ± 1.2 mg/g tissue, $P < .05$). However, pair-fed controls exhibited a twofold higher TG content than vehicle controls (12.2 ± 1.1 *v* 6.1 ± 1.2 mg/g tissue, $P < .05$). The TG content of the heart did not change significantly among the three groups (Fig 4).

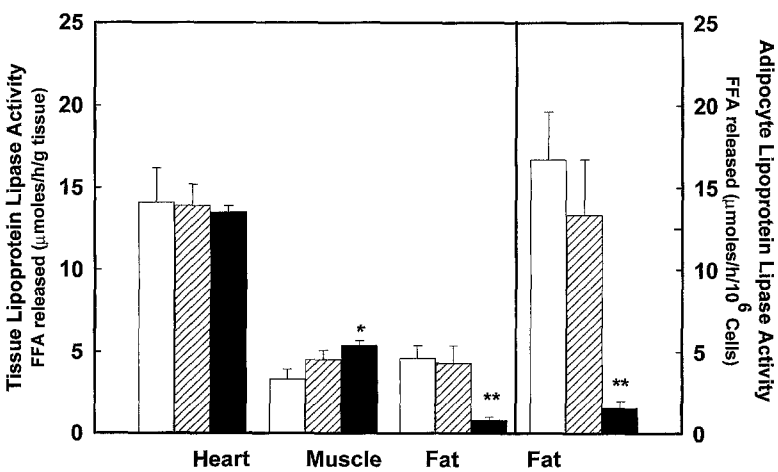

Fig 3. Effects of BC/SKF treatment on LPL activities. Heart, parametrial adipose tissue, and soleus muscle pairs were collected 24 hours after the final injection from control (□), pair-fed (▨), and BC/SKF mice (■). Results are the mean ± SE of 6-8 mice per group. *$P < .05$ and **$P < .01$ *v* controls.

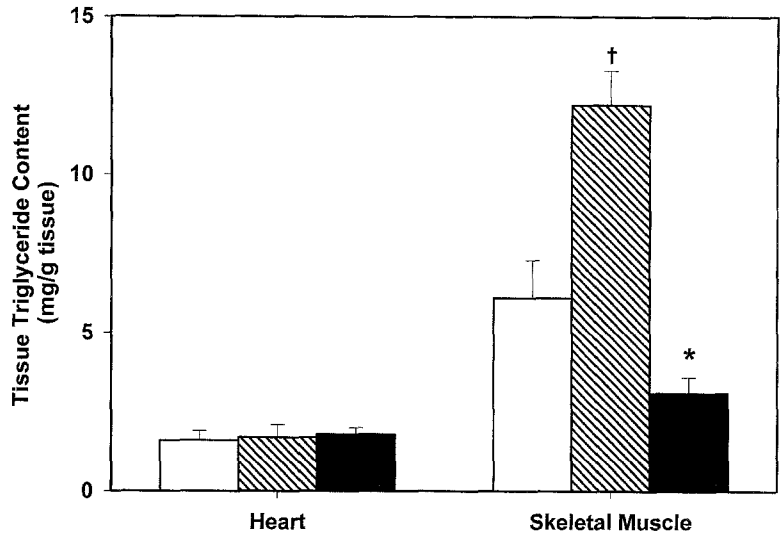

Fig 4. Effects of BC/SKF treatment on TG content in heart and skeletal muscle tissue. Heart and skeletal muscle were collected from control (□), pair-fed (▨), or BC/SKF mice (■), and TG in these tissues was extracted. Results are the mean ± SE of 6-8 mice per group. *$P < .01$ *v* controls. †$P < .01$ *v* control and BC/SKF.

Effects of BC/SKF on Lipolysis in Parametrial Adipose Tissue

Basal and 0.1 and 1.0 μmol/L isoproterenol–stimulated rates of lipolysis in adipose tissue from control and pair-fed ob/ob mice were 0.66 ± 0.07 and 0.91 ± 0.07 ($P < .05$), 1.07 ± 0.15 and 1.64 ± 0.21 ($P = .06$), and 1.33 ± 0.22 and 2.35 ± 0.2 ($P < .01$) pmol glycerol/h/cell, respectively. However, BC/SKF treatment reduced lipolysis, as opposed to the increase induced by pair-feeding. The basal lipolytic rate in BC/SKF-treated animals was 0.31 ± 0.07 pmol glycerol/h/cell, which was 53% and 66% less than the rate in control and pair-fed groups, respectively ($P < .01$). BC/SKF treatment also reduced isoproterenol (0.1 and 1 μmol/L)-stimulated lipolysis 50% to 56% ($P < .01$) relative to vehicle controls and 66% to 75% ($P < .01$) relative to pair-fed controls (Fig 5).

DISCUSSION

These studies clearly demonstrate that treatment of hyperlipidemic and hyperglycemic ob/ob mice with the dopamine D_2/D_1 agonist combination BC/SKF substantially reduces plasma levels of chylomicron-TG and VLDL-TG. Total plasma TG levels represent a balance between intestinal absorption and hepatic synthesis and secretion versus TG clearance into the tissues. The reduction of plasma chylomicron-TG levels may be ascribed, in part, to the normalization of hyperphagia, and the reduction of plasma VLDL-TG, to the reduced hepatic synthesis and secretion of VLDL-TG. We have previously shown that BC/SKF reduces hepatic lipogenic enzyme activities independent of its effects to normalize hyperphagia in ob/ob mice.[23] The present studies demonstrate that such treatment reduces hepatic lipid (re)esterification and synthesis as measured by ^{3}H-glycerol incorporation into triacylglycerol. Furthermore, the hepatic VLDL-TG secretion rate was reduced 40% by BC/SKF treatment. Therefore, the decrease of hepatic TG synthesis and secretion is a contributing factor to the treatment-induced reduction in plasma VLDL-TG (and phospholipid) levels observed. This reduced hepatic activity may be due in part to the reduction of chronic hyperinsulinemia[38] in these treated mice. Moreover, the reduction of neither chylomicron-TG nor VLDL-TG by BC/SKF treatment can be attributed to increased transport of these lipoprotein TGs into adipose tissue, inasmuch as adipose LPL activity was dramatically reduced by treatment. Adipose LPL activity is elevated in ob/ob versus lean mice,[17] and the normalization of its activity by BC/SKF treatment may contribute to the reduction in the serum FFA level. The reduced serum FFA, in turn, may contribute to a reduced hepatic reesterification to VLDL-TG as suggested by our ^{3}H-glycerol

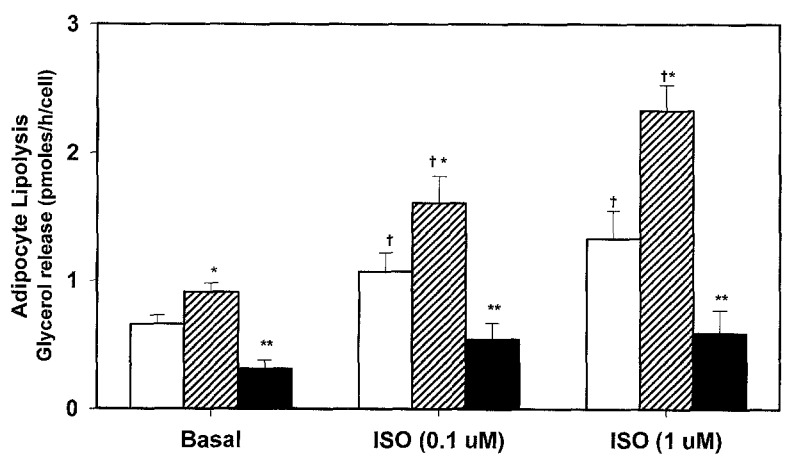

Fig 5. Effects of BC/SKF treatment on lipolysis in adipose tissue. Parametrial adipose tissue was collected from control (□), pair-fed (▨), and BC/SKF mice (■), and adipose cells were isolated by a collagenase digestion method. Results are the mean ± SE of 6-8 mice per group. *$P < .05$ *v* controls. **$P < .01$ *v* control and pair-fed groups. †$P < .01$ *v* respective basal lipolysis. ISO, isoproterenol.

incorporation data. Although muscle LPL activity was increased by dopamine agonist treatment, the smaller muscle mass relative to adipose tissue in these mice coupled with the much more dramatic decrease of adipose LPL activity relative to the modest increase in muscle LPL activity (Fig 3) argue strongly against the decreased plasma VLDL-TG resulting from overall increased clearance.

The observed reduction in adipose lipolytic activity following treatment may further contribute to reduced hepatic VLDL-TG synthesis and secretion by reducing the circulating levels of the substrate, FFA. BC/SKF treatment reduced the basal and isoproterenol-stimulated lipolytic rate by approximately 50%. The 50% reduction of basal lipolysis among treated versus control animals was associated with a nearly equivalent reduction in circulating FFA levels. The 50% reduction in caloric intake concurrent with a 50% reduction in lipolysis suggests that a new metabolic steady state is instated with this treatment. If the caloric intake is perceived as a perturbation from normal (ie, starvation), then one would expect to see compensatory increases in lipolysis, as are in fact observed in pair-fed animals (Fig 5). Contrariwise, if the reduced caloric intake is one aspect of an overall response to a new regulatory signal initiating a normalization of hyperphagia and metabolism, then one would expect to see a reduction of lipolysis and serum FFA toward levels observed in lean mice, as in the case with BC/SKF treatment. Consequently, lipogenesis is reduced due to reduced feeding and reduced lipogenic activity in liver and adipose tissue,[23] and adipose lipolysis is likewise reduced to a similar extent, following 2 weeks of BC/SKF treatment.

Skeletal muscle TG content was markedly reduced following BC/SKF treatment. The skeletal muscle TG pool is the most labile total body fat component. It is greatly affected by energy intake, energy expenditure, and glucose homeostasis.[39] The muscle TG content may be reduced as a function of decreased substrate delivery (ie, plasma lipoprotein-TG and plasma FFA) and/or increased tissue utilization (oxidation). A myriad of physiologic and pathologic factors (including muscle type, oxidative or glycolytic) interact to regulate these two biological processes.

Considering substrate delivery, increases in serum TG and/or FFA levels are positively correlated with increases in muscle TG content in animals and humans.[40,41] Also, reducing serum lipid content reduces muscle TG levels.[41] As much as 70% to 90% of nonesterified fatty acids taken up by resting muscle can be rapidly esterified and incorporated into lipid droplets depending on the existing physiologic circumstance.[42] Hyperglycemia also induces increases in muscle TG content,[43] partly because blood glucose is the major source of the α-glycerol-phosphate backbone of TG. As such, the large reduction in serum FFA and lipoprotein-TG, as well as glucose, may contribute to the reduced muscle TG content of BC/SKF-treated animals. In support of this conclusion is the fact that among pair-fed animals, increases in lipolytic activity and serum FFA are positively correlated with large increases in muscle TG content compared with controls (Fig 4). However, a reduced fat oxidation rate in response to the hypocaloric (ob/ob) diet may also contribute to elevated muscle TG levels[44] in these pair-fed mice.

Whether muscle fatty acid oxidation is increased, decreased, or unaffected in absolute terms by BC/SKF treatment has not yet been determined. However, increased substrate availability is a primary determinant of fatty acid oxidation,[45,46] and increased circulating FFA levels, characteristic of obesity,[47,48] are positively correlated with increases in fatty acid oxidation.[47,48] Nonetheless, the possibility still exists that BC/SKF treatment may maintain elevated muscle fatty acid oxidation relative to the prevailing serum lipid concentration (ie, no decrease in absolute oxidation rate to equilibrate with the decreased serum lipid delivery), and we are currently exploring this possibility. Additionally, a BC/SKF-induced reduction in muscle FFA reesterification to triacylglycerol within the muscle cell may also contribute to a decreased intracellular TG pool size. Our preliminary data with muscle cell homogenates suggest that BC/SKF treatment of ob/ob mice may reduce the FFA reesterification capacity of skeletal muscle.

The intracellular TG pool is a source for FFA oxidation and long-chain fatty acyl coenzyme A, and increases in both can induce insulin resistance in muscle.[49] Therefore, the effects of BC/SKF to reduce muscle TG may contribute to an improvement in insulin sensitivity as evidenced by decreases in plasma glucose and insulin. There are now several reports of an association between increased muscle TG levels and insulin resistance.[50] Compared with healthy subjects, a sixfold increase of muscle TG content was found in patients with non–insulin-dependent diabetes mellitus.[21] Furthermore, in nondiabetic humans, the skeletal muscle TG level was found to be inversely correlated with insulin action.[51] In rats, a high-fat diet causes insulin resistance, the degree of which is related to the resultant TG content of muscle.[22] Finally, as it relates to these studies, muscles of ob/ob mice, which are markedly insulin-resistant, have a threefold to fourfold increase in TG content relative to TG content in lean mice.[20] The possible mechanisms by which increased muscle intracellular levels of TG induce cellular resistance to insulin-stimulated nonoxidative glucose disposal are not completely understood.[49]

The mechanism(s) by which BC/SKF treatment induces metabolic effects are under investigation. The BC/SKF-induced normalization of hyperphagia in these ob/ob mice accounts in large part for the reduction in BW gain, but not for the reductions in serum glucose, TG, or FFA levels, muscle TG content, adipose LPL activity or lipolysis, parametrial adipocyte cell size (present study), or lipogenic enzyme activities.[23] The establishment of a new steady state of reduced lipid synthesis/storage and mobilization coupled with reduced hyperglycemia and hyperinsulinemia may occur via influences on the neuroendocrine axis. Dopamine agonist treatment of these mice has been shown to reduce elevated hypothalamic neuropeptide Y and serum corticosterone and glucagon levels,[23,52,53] which all potentiate the obese insulin-resistant state.[54-56] BC reduces elevated levels of norepinephrine and serotonin within the ventromedial hypothalamus of insulin-resistant hamsters,[57] and such action may be expected to reduce insulin resistance[57,58] and dyslipidemia.[59] Interestingly, several animal models of obesity/diabetes show decreased dopaminergic and increased

noradrenergic/serotonergic activities within metabolic centers of the CNS.[57] BC/SKF treatment may be expected to increase the dopamine to norepinephine/serotonin ratio centrally (and peripherally) and thereby elicit its metabolic effects.[60]

In conclusion, the effect of BC/SKF to ameliorate dyslipidemia in ob/ob mice likely reflects its simultaneous antilipogenic action in liver and adipose tissue and antilipolytic action in adipose tissue. Decreased plasma flux of FFA leads to a smaller precursor pool for hepatic synthesis and secretion of VLDL-TG. The concurrent inhibition of LPL activity in adipose tissue by BC/SKF also contributes to the decreased plasma FFA level. The decreasing levels of circulating FFA and lipoprotein-TG may contribute to the decreased TG levels in muscle and thereby improve insulin sensitivity in this tissue of BC/SKF-treated ob/ob mice. Such treatment may have a therapeutic benefit in reducing hypertriglyceridemia (and the associated cardiovascular risk) in type 2 diabetes. BC/SKF treatment reduces fat in the body (adipose storage and muscle), in the blood, and in the diet (ie, chylomicrons, by virtue of its ability to normalize hyperphagia).

REFERENCES

1. Howard BV, Howard WJ Dyslipidemia in non–insulin-dependent diabetes mellitus. Endocr Rev 15:263-274, 1994
2. Steiner G: Dyslipoproteinemias in diabetes. Clin Invest Med 18:282-287, 1995
3 Yoshino G, Hirano T, Kazumi T: Dyslipidemia in diabetes mellitus. Diabetes Res Clin Pract 33:1-14, 1996
4. Hokanson JE, Austin MA: Plasma triglyeride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: A meta-analysis of population-based prospective studies. J Cardiovasc Risk 3:213-219, 1996
5. Gaziano JM, Hennekens CH, O'Donnell CJ, et al: Fasting triglycerides, high-density lipoprotein, and risk of myocardial infarction. Circulation 96:2520-2525, 1997
6. Patsch JR, Miesenbock G, Hopferwieser T, et al: Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. Arterioscler Thromb 12:1336-1345, 1992
7. Boden G, Chen X, Ruiz J, et al: Mechanisms of fatty acid–induced inhibition of glucose intake. J Clin Invest 94:2349-2356, 1994
8. Boden G: Fatty acids and insulin resistance. Diabetes Care 19:394-395, 1996
9. Kruszynska YT: The role of fatty acid metabolism in the hypertriglyceridemia and insulin resistance of type 2 (non–insulin dependent) diabetes. Diabetes Ann 9:107-139, 1995
10. Kruszynska YT, Mulford MI, Yu JG, et al: Effects of nonesterified fatty acids on glucose metabolism after glucose ingestion. Diabetes 46:1586-1593, 1997
11. Meier AH, Cincotta AH: Circadian rhythms regulate the expression of the thrifty genotype/phenotype. Diabetes Rev 4:464-487, 1996
12. Cincotta AH, Schiller BC, Meier AH: Bromocriptine inhibits the seasonally occurring obesity, hyperinsulinemia, insulin resistance, and impaired glucose tolerance in the Syrian hamster, *Mesocricetus auratus*. Metabolism 40:639-644, 1991
13. Cincotta AH, MacEachern TA, Meier AH: Bromocriptine redirects metabolism and prevents seasonal onset of obese hyperinsulinemic state in Syrian hamsters. Am J Physiol 264:E285-E293, 1993
14. Cincotta AH, Meier AH: Bromocriptine inhibits in vivo free fatty acid oxidation and hepatic glucose output in seasonally obese hamsters (*Mesocricetus auratus*). Metabolism 44:1349-1355, 1995
15. Hems DA, Rath EA, Verrinder TR: Fatty acid synthesis in liver and adipose tissue of normal and genetically obese (ob/ob) mice during the 24-hour cycle. Biochem J 150:167-173, 1975
16. Cheema SK, Clandinin MT: Diet fat alters expression of genes for enzymes of lipogenesis in lean and obese mice. Biochim Biophys Acta 1299:284-288, 1996
17. Gasquet PD, Pequignot E: Lipoprotein lipase activities in adipose tissues, heart and diaphragm of the genetically obese mouse (ob-ob). Biochem J 127:445-447, 1972
18. Nishina PM, Lowe S, Wang J, et al: Characterization of plasma lipids in genetically obese mice: The mutants obese, diabetes, fat, tubby, and lethal yellow. Metabolism 43:549-553, 1994
19. Stevenson RW, Hutson NJ, Krupp MN, et al: Actions of novel antidiabetic agent englitazone in hyperglycemic hyperinsulinemic ob/ob mice. Diabetes 39:1218-1227, 1990
20. Trostler N, Amin R, Shafrir E: Increased protease activity in muscles of obese (ob/ob) mice. Int J Obes 6:557-566, 1982
21. Falholt K, Jensen I, Lindkaer JS, et al: Carbohydrate and lipid metabolism in biopsies from the aorta in patients undergoing coronary bypass surgery. Diabet Med 5:27-31, 1988
22. Storlien LH, Jenkins AB, Chisholm DJ, et al: Influence of dietary fat composition on development of insulin resistance in rats. Relation to muscle triglyceride and w-3 fatty acids in muscle phospholipids. Diabetes 40:280-289, 1991
23. Scislowski PWD, Tozzo E, Zhang Y, et al: Attenuation of diabetic and obese conditions in ob/ob mice treated with dopaminergic agonists. Int J Obes 23:425-431, 1999
24. Blight EG, Dyer WJ: A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911-917, 1959
25. Nikkila EA, Kekki M: Polymorphism of plasma triglyceride kinetics in normal human adult subjects. Acta Med Scand 190:49-59, 1971
26. Kasim SE, LeBoeuf RC, Khilnani S, et al: Mechanisms of triglyceride-lowering effect of an HMG-CoA reductase inhibitor in a hypertriglyceridemic animal model, the Zucker obese rat. J Lipid Res 33:1-7, 1992
27. Bannerman RM: in Foster HL, Small JD, Fox JG (eds): The Mouse in Biomedical Research. New York, NY, Academic, 1983, pp 294-312
28. Havel RJ, Eder HA, Bragdon JH: The distribution and chemical composition of ultracentrifugally-separated lipoproteins in human serum. J Clin Invest 34:1345-1353, 1955
29. Gerdes LU, Gerdes C, Klausen IC, et al: Generation of analytic plasma lipoprotein profiles using two prepacked Superose 6B columns. Clin Chim Acta 205:1-9, 1992
30. Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the Folin phenol reagent. J Biol Chem 193:339-358, 1951
31. Hietanen E, Greenwood MR: A comparison of lipoprotein lipase activity and adipocyte differentiation in growing male rats. J Lipid Res 18:480-490, 1977
32. Belfrage P, Vaughan M: Simple liquid-liquid partition system for isolation of labeled oleic acid from mixtures with glycerides. J Lipid Res 10:341-344, 1969
33. Honnor RC, Dhillon GS, Londos C: cAMP-dependent protein kinase and lipolysis in rat adipocytes. J Biol Chem 260:15122-15129, 1985
34. Lavau M, Susini C, Knittle J, et al: A reliable photomicrographic method to determine fat cell size and number. Application to dietary obesity. Proc Soc Exp Biol Med 156:251-256, 1977
35. Cushman SW, Salans LB: Determinations of adipose cell size and number in suspensions of isolated rat and human adipose cells. J Lipid Res 19:269-273, 1978

36. Tozzo E, Gnudi L, Kahn BB: Amelioration of insulin resistance in streptozotocin diabetic mice by transgenic overexpression of GLUT4 driven by an adipose-specific promoter. Endocrinology 138:1604-1611, 1997

37. Camus MC, Aubert R, Bourgeois F, et al: Serum lipoprotein and apolipoprotein profiles of the genetically obese ob/ob mouse. Biochim Biophys Acta 961:53-64, 1988

38. Steiner G, Lewis GF: Hyperinsulinemia and triglyceride-rich lipoproteins. Diabetes 45:S24-S26, 1995 (suppl)

39. Comizio R, Pietrobelli A, Tan YX, et al: Total body lipid and triglyceride response to energy defect: Relevance to body composition models. Am J Physiol 274:E860-E866, 1998

40. Phillips DI, Caddy S, Ilic V, et al: Intramuscular triglyceride and muscle insulin sensitivity: Evidence for a relationship in nondiabetic subjects. Metabolism 45:914-950, 1996

41. Oakes ND, Bell KS, Furler SM, et al: Diet-induced muscle insulin resistance in rats is ameliorated by acute dietary lipid withdrawal or a single bout of exercise: Parallel relationship between insulin stimulation of glucose uptake and suppression of long-chain fatty acyl-CoA. Diabetes 46:2022-2028, 1997

42. Oscai LB, Essig DA, Plamer WK: Lipase regulation of muscle triglyceride hydrolysis. J Appl Physiol 69:1571-1577, 1990

43. Laybutt DR, Chisholm DJ, Kraegen EW: Specific adaptations in muscle and adipose tissue in response to chronic systemic glucose oversupply in rats. Am J Physiol 237:E1-E9, 1997

44. Ardawi MS, Majzoub MF, Masoud IM, et al: Enzymatic and metabolic adaptations in the gastrocnemius, plantaris and soleus muscles of hypocaloric rats. Biochem J 261:219-225, 1989

45. Dagenais GR, Tancredi RG, Zierler KL: Free fatty acid oxidation by forearm muscle at rest, and evidence for an intramuscular lipid pool in the human forearm. J Clin Invest 58:421-431, 1976

46. Sidossis LS, Wolfe RR: Glucose and insulin-induced inhibition of fatty acid oxidation: The glucose-fatty acid cycle reversed. Am J Physiol 270:E733-E738, 1996

47. Groop LC, Bonadonna RC, DelPrato P, et al: Glucose and free fatty acid metabolism in non–insulin dependent diabetes mellitus. Evidenced for multiple sites of insulin resistance. J Clin Invest 84:205-213, 1989

48. Golay A, Chen YD, Reaven GM: Effect of differences in glucose tolerance on insulin's ability to regulate carbohydrate and free fatty acid metabolism in obese individuals. J Clin Endocrinol Metab 62:1081-1088, 1986

49. Ruderman NB, Saha AK, Vavvas D, et al: Malonyl CoA, fuel sensing and insulin resistance. J Physiol (in press)

50. Shimabukuro M, Koyama K, Chen G, et al: Direct antidiabetic effect of leptin though triglyceride depletion of tissues. Proc Natl Acad Sci USA 94:4637-4641, 1997

51. Pan DA, Lillioja S, Kriketos AD, et al: Skeletal muscle triglyceride levels are inversely related to insulin action. Diabetes 46:983-988, 1997

52. Bina K, Hodge S: Treatment with dopaminergic D1/D2 agonists in the ob/ob mouse decreases hyperphagia, hyperglycemia, obesity and NPY immunoreactivity in discrete hypothalamic nuclei. Diabetes 46:228A, 1997 (abstr)

53. Liang Y, Lubkin M, Sheng H, et al: Dopamine agonist treatment ameliorates hyperglycemia, hyperlipidemia, and the elevated basal insulin release from islets of ob/ob mice. Biochim Biophys Acta 1405:1-13, 1999

54. Feldkircher KM, Mistry AM, Romsos DR: Adrenelectomy reverses pre-existing obesity in adult genetically obese (ob/ob) mice. Int J Obes 20:232-235, 1996

55. Nagai K, Nakagawa H (eds): Central Regulation of Energy Metabolism With Special Reference to Circadian Rhythm. Boca Raton, FL, CRC, 1992

56. Chen HL, Romsos DR: Dexamethasone rapidly increases hypothalamic neuropeptide Y secretion in adrenalectomized ob/ob mice. Am J Physiol 271:E151-E158, 1996

57. Luo S, Meier AH, Cincotta AH: Bromocriptine reduces obesity, glucose intolerance and extracellular monoamine metabolite levels in the ventromedial hypothalamus of Syrian hamsters. Neuroendocrinology 68:1-10, 1998

58. Shimazu T, Ishikawa K: Modulation by the hypothalamus of glucagon and insulin secretion in rabbits: Studies with electrical and chemical stimulation. Endocrinology 108:605-611, 1981

59. Zhang Y, Prevelige R, Luo J, et al: Chronic infusion of norepinephrine into the ventromedial hypothalamus induces dyslipidemia and hyperinsulinemia in Syrian hamsters. Diabetes 47:298A, 1998 (abstr)

60. Luo S, Liang Y, Cincotta AH: Intracerebroventricular administration of bromocriptine ameliorates the insulin-resistant/glucose-intolerant state in hamsters. Neuroendocrinology 69:160-166, 1999