Changes in Dietary Fatty Acids Modify the Decreased Lipolytic β_3-Adrenergic Response to Hyperinsulinemia in Adipocytes From Pregnant and Nonpregnant Rats

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The effect of dietary olive oil and fish oil on the lipolytic dose-response of the β_3-adrenergic agonists, epinephrine, isoproterenol, BRL-37344, and CGP-12177, in adipocytes was studied in pregnant and virgin rats either untreated or under hyperinsulinemic-euglycemic conditions. Rats were fed a semisynthetic diet containing 5% of either olive oil or fish oil and studied at day 20 of treatment and/or gestation. Plasma glucose was lower and plasma insulin, triglycerides, and free fatty acids (FFAs) were higher in pregnant versus virgin rats, and the insulin sensitivity index was lower in the former. Lumbar adipose tissue phospholipid fatty acids showed a significantly higher monounsaturated fatty acid and a lower (n − 3) fatty acid content in rats fed the olive oil diet versus the fish oil diet. The lipolytic dose-response curve of either adrenergic agent was always lower in adipocytes from untreated pregnant rats versus virgin rats, and whereas the hyperinsulinemic-euglycemic clamp decreased these responses in adipocytes from virgin rats fed the olive oil diet only, adipocytes from pregnant rats always showed a decreased dose-response lipolytic curve. Thus, the lipolytic responsiveness of β_3-adrenoceptor (β_3-AR) agonists by adipocytes is impaired in cells from rats made hyperinsulinemic chronically by pregnancy or acutely by the hyperinsulinemic-euglycemic clamp, but such response to the acute condition disappears when the adipocyte phospholipid composition is modified by changes in dietary fatty acids.

ALTHOUGH DURING PREGNANCY the mother has a net accumulation of fat depots as shown both in women[1,2] and in rats,[3-5] during late pregnancy there is an enhancement in adipose tissue lipolysis,[6-9] which seems to be caused by the maternal insulin resistance condition.[10,11]

Enhanced maternal adipose tissue lipolytic activity during late gestation has also been related to the autonomous nervous system activation, secondary to hypoglycemia.[9,12,13] However, a decreased response of adipocytes to noradrenaline or isoproterenol has been reported during late gestation in rats[14] and in sheep.[15,16] This effect could be a consequence of a decrease in adrenoceptor activity secondary to maternal hyperinsulinemia and insulin resistance, since insulin has been shown to downregulate β_3-adrenergic receptor (β_3-AR) expression[17] and to translocate β-ARs from the outside to the inside of the fat cell.[18] Also, conditions of insulin resistance have been shown to reduce the expression of β_2-ARs, causing a resistance to catecholamine.[19,20]

The existence of 3 β-AR (β_1-, β_2-, and β_3-AR) subtypes in rat adipocytes is now accepted, and these receptors share the same physiologic catecholamines, norepinephrine and epinephrine.[21] Besides, it has also been shown that an increase in saturated fatty acids in the composition of the diet decreases β-AR binding, resulting in a lower lipolytic activity in rat adipose tissue.[22] This effect has been proposed to contribute to the body fat accumulation associated with a rich saturated fatty acid intake as compared with a rich monounsaturated or n-3 polyunsaturated fatty acid intake.[23] In addition, insulin action in adipocytes from insulin-resistant rats correlates with the fatty acid unsaturation index in membrane phospholipids,[24] indicating that dietary intervention may play a key role in the sensitivity of the tissue to hormones.

The β_3-AR plays a key role in the regulation of lipolysis in rodent white adipose tissue,[17,25-27] and the effect of insulin on this receptor has been proposed to represent a newly characterized and potent mechanism to modulate all cyclic adenosine monophosphate–dependent biologic processes.[17] The present study was addressed to test the physiologic relevance of such a mechanism in an ex vivo/in vivo condition, and for this purpose, 3 specific questions were analyzed. First, the existence of differences in the lipolytic responsiveness to β_3-AR agonists between white adipose tissue cells from pregnant rats, which have hyperinsulinemia and insulin resistance, and adipocytes from virgin rats was tested. Second, the potential modifications of such responsiveness in pregnant and virgin rats in a condition of acute hyperinsulinemia such as that caused by a hyperinsulinemic-euglycemic clamp were studied. And third, we also examined whether changes in dietary fats modify β_3-AR responsiveness in adipocytes from pregnant and virgin rats.

MATERIALS AND METHODS

All procedures involving animals were approved by the Animal Research Committee of the Faculty of Experimental Sciences, Universidad San Pablo-CEU, Spain.

Animals and Diets

Female Sprague-Dawley rats initially weighing 180 to 200 g from our own animal quarters were kept under controlled temperature (22° ± 1°C), humidity, air flow, and a 12-hour light-dark cycle (8 AM to 8 PM). Rats were randomly selected, and half of them were mated with males of the same strain. The day on which spermatozoids appeared in the vaginal smear was considered day 0 of gestation, and from that time on, rats were fed ad libitum with one of the semipurified diets (Table 1). The 2 isoenergetic diets (15.28 kJ/g) had a paste structure and were

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Table 1. Composition of the Experimental Diets (g/kg diet)

Ingredient	Olive Oil Diet	Fish Oil Diet
Casein	170	170
Salt mix*	35	35
Vitamin mix†	10	10
Choline chloride	2	2
DL-Methionine	3	3
Cellulose	100	100
Cornstarch	630	630
Olive oil	50	
Fish oil		50

NOTE. Composition is based on the AIN-76A diet.

*Salt mix (g/kg diet): dibasic calcium phosphate 17.5, magnesium oxide 0.84, potassium citrate monohydrate 7.7, potassium sulfate 1.82, sodium chloride 25.9, chromium potassium sulfate 0.019, cupric carbonate 0.05, potassium iodate 0.0003, ferric citrate 2.1, manganese carbonate 0.1225, sodium selenite 0.0003, and zinc carbonate 0.056.

†Vitamin mix (mg/kg diet): retinyl palmitate 2.4, cholecalciferol 0.025, DL-α-tocopheryl acetate, 50.0, menadione sodium bisulfite 0.8, biotin 0.22, cyanocobalamin 0.01, riboflavin 6.6, and thiamine hydrochloride 6.6.

prepared twice weekly and kept refrigerated at 4°C. The only difference was the fat composition, either 5% olive oil or fish oil. The fatty acid composition of the 2 diets was analyzed in lipid extracts[28] after hydrolysis and methylation using a gas-liquid chromatograph system (Perkin Elmer Autosystem, Norwalk, CT; provided with a coated fused-silica capillary column, 0.25 mm ID). The fatty acid composition of the 2 diets is shown in Table 2. Pregnant and virgin rats were housed in collective polycarbonate cages (4 rats per cage) with wood chip bedding, and body weight and food intake were measured 3 times per week. On day 20 of gestation and feeding the corresponding diet, half of the virgin and pregnant rats were subjected to a hyperinsulinemic clamp as described later, and all animals were decapitated between 10 AM and 12 noon. Lumbar fat pads were rapidly removed, and 1 aliquot was immediately placed in liquid nitrogen for lipid analysis and the remaining tissue was placed in saline at room temperature.

Fatty Acid Composition of Adipocyte Phospholipids

Total lipids were extracted from the frozen aliquot of the lumbar fat pads,[28] and neutral lipids and phospholipids were separated on thin-layer chromatography in silica-gel 60 F_{254}.[29] Spots corresponding to phospholipids were eluted with methanol:toluene (4:1), and after transmethylation, fatty acid methyl esters were analyzed on a gas-liquid chromatograph.

Table 2. Fatty Acid Composition of Olive Oil and Fish Oil Diets Analyzed With Gas-Liquid Chromatography (g/100 g fatty acids)

Fatty Acid	Olive Oil Diet	Fish Oil Diet
12:0	1.39	0.01
14:0	1.16	5.49
15:0	0.29	0.01
16:0	14.3	14.4
18:0	5.32	4.19
22:0	0.10	0.01
26:0	0.34	0.01
16:1 (n-7)	1.49	5.03
18:1 (n-9)	65.9	18.3
20:1 (n-9)	0.01	6.27
22:1 (n-9)	1.46	10.0
18:2 (n-6)	7.39	2.02
18:3 (n-3)	0.67	3.63
20:5 (n-3)	0.15	9.37
22:6 (n-3)	0.01	8.98

Hyperinsulinemic-Euglycemic Clamps

Some rats from each group were anesthetized with an intraperitoneal injection of 2.5 mL/kg of a cocktail containing ketamine (57.6 mg/mL), diazepam (10 mg/mL), and atropine (1 mg/mL), and indwelling catheters were inserted into the left jugular vein and the right carotid artery. Human insulin (Actrapid; Novo, Copenhagen, Denmark) was infused through the jugular vein at a constant rate of 0.4 U/h/kg body weight. Blood samples were withdrawn from the carotid artery every 5 minutes during the first hour, and the blood glucose concentration was determined using a glucose analyzer (Accutrend; Boehringer, Mannheim, Germany). Blood glucose was maintained at euglycemic levels with a variable infusion of glucose (20% wt/vol) started 1 minute after beginning the insulin infusion. Blood samples were centrifuged at 4°C, and the plasma was frozen at −20°C for further insulin analysis with a rat specific radioimmunoassay kit (Incstar, Minneapolis, MN). At the end of the 60 minutes, the rats were decapitated and the lumbar fat pads were removed and placed in saline at room temperature. The glucose disposal rate was calculated as a function of the rate of glucose infusion at steady state corrected by body weight. The insulin sensitivity index (Sip) was calculated as the ratio of the rate of glucose infusion at steady state and the blood glucose concentration times the increase in plasma insulin during the clamp, corrected by body weight, as before.[10]

Preparation and Incubation of Adipocytes

Adipocytes were isolated from lumbar fat pad tissue by collagenase digestion at 37°C with shaking (1 mg/mL collagenase A; Boehringer) in Krebs Ringer bicarbonate buffer, pH 7.4, containing 4% bovine serum albumin ([BSA] fraction V, essentially free of free fatty acids [FFAs]; Sigma, St Louis, MO) and 5.5 mmol/L glucose according to the method of Rodbell.[30]

Lipolytic Studies

Adipocytes (5.58 ± 0.31 mg tissue lipids) were incubated in 0.5 mL Krebs Ringer bicarbonate buffer, pH 7.4, supplemented with 4% BSA (essentially free of FFAs), glucose (5.5 mmol/L), and adenosine desaminase (1 U/mL), as well as increasing amounts (10^{-9} to 10^{-5} mol/L) of either epinephrine bitartrate (Sigma), isoproterenol (Sigma), BRL-37344 (kindly provided by Beecham, Epson, UK), or CGP-12177 (kindly provided by Ciba-Geigy, Madrid, Spain). Isoproterenol is a nonselective β-agonist, whereas BRL-37344 is a β_3-AR–selective agonist that also activates β_1- and β_2-ARs, although with a lower potency,[31-33] and CGP-12177 is a β_3-AR agonist but also a β_1/β_2-AR antagonist,[33] and thus allows specific coupling of β_3-AR to the adenylate cyclase system. After a 90-minute incubation at 37°C under a 95% O_2/5% CO_2 gas phase and shaking (80 cycles/min), the reaction was stopped by dipping the tubes in an ice bath. After centrifugation (2,000 × *g* at 4°C for 10 minutes), an aliquot of the infranatant was taken to enzymatically evaluate glycerol release during the incubation,[34] expressed per 100 mg lipids. Concentration-response curves of glycerol release as a function of either agent were calculated as a percentage of the values obtained in the absence of the corresponding agent and analyzed by computer-assisted iteration using Graph-PAD Software (Instat, San Diego, CA).

Statistical Analysis

Results are expressed as the mean ± SEM. ANOVA was used to treat the significance of differences between dietary groups. The statistical significance of differences was tested by Tukey's test, and linear regressions were calculated by the least-squares method. Differences between 2 groups were analyzed by Student's unpaired test.

The concentration-response curves were fitted to Hill's model by

computer analysis (Sigma Plot 4.0) for the estimation of potencies (50% effective concentration [EC_{50}] values).

RESULTS

At the beginning of the experiments, there were no differences in the body weight of the rats, and although pregnant rats gained more weight than virgins during the 20 days of pregnancy and on either specific diet, the weight gain was not different between the 2 diets in either virgin or pregnant rats. The diets also did not affect the pregnancy outcome, as shown by the litter size or average fetal or placental weight (Table 3).

Lumbar fat pad weight was similar in rats fed the fish oil diet and olive oil diet, virgin or pregnant. However, this variable was always higher in pregnant versus virgin rats, and the differences were statistically significant between pregnant and virgin rats (Table 4).

Blood glucose levels were lower in pregnant rats than in virgin rats, whereas plasma insulin levels were higher in the former, and no differences due to the diet were detected in any of the groups. Both plasma FFA and triglyceride levels were always higher in pregnant versus virgin rats, although the dietary interventions did not affect these variables in either group (Table 5).

Fatty Acid Composition of Adipose Tissue Phospholipids

The fatty acid profiles of lumbar adipose tissue phospholipids were similar for pregnant and virgin rats fed either diet, except for a higher (n-6)/(n-3) fatty acid ratio in the former when fed the olive oil diet. This fatty acid profile in adipose tissue phospholipids reflects the fatty acid composition of the 2 diets: the proportion of oleic and linoleic acids and total monounsaturated, total (n-6), and (n-6)/(n-3) were higher in the olive oil–fed rats, whereas the proportion of EPA (20:5, n-3) and DHA (22:6, n-3) and total (n-3) fatty acids were significantly lower in olive oil–fed rats versus the fish oil group (Table 6).

Hyperinsulinemic-Euglycemic Clamp

Some rats were subjected to a hyperinsulinemic-euglycemic clamp with 0.4 IU insulin/h/kg for 60 minutes. Blood glucose remained constant during the clamp, with pregnant rats having lower levels than virgin rats (Table 7). However, plasma insulin increased in both pregnant and virgin rats (Table 7) as compared with basal values (Table 5), and although the values attained under the hyperinsulinemic-euglycemic clamp condition in the former were always higher versus the latter, they were not significantly different as a consequence of the high variation within each group. However, the glucose disposal rate and insulin sensitivity index were significantly lower in pregnant versus virgin rats, although no effect of dietary composition was found for any of these variables related to insulin sensitivity in either pregnant or virgin rats (Table 7).

Table 3. Effect of Olive Oil and Fish Oil Diets on Body Weight in Virgin and Pregnant Rats and Fetal and Placental Weight

Parameter	Olive Oil Diet	Fish Oil Diet
Virgin rats		
Initial body weight (g)	193 ± 3	184 ± 3
Final body weight (g)	249 ± 4	235 ± 9
Pregnant rats		
Initial body weight (g)	187 ± 4	186 ± 4
Final body weight (g)	267 ± 9	259 ± 7
Fetus body weight (g)	4.00 ± 0.26	3.61 ± 0.08
Placenta weight (g)	0.55 ± 0.04	0.58 ± 0.03
Fetuses (n per dam)	12.8 ± 1.2	15.0 ± 0.8

NOTE. Results are the mean ± SE. Mean values between the 2 groups were not significant ($P > .05$) for any of the variables (n = 5-6 rats per group).

Table 4. Effect of Olive Oil and Fish Oil Diets on Lumbar Fat Pad Weight in Virgin and Pregnant Rats

Group	Olive Oil Diet	Fish Oil Diet
Virgin	1.16 ± 0.07[a]	0.82 ± 0.07[a]
Pregnant	1.65 ± 0.10[b]	1.35 ± 0.10[b]

NOTE. Results are the mean ± SE. Values are expressed as grams of total lumbar fat pad. Statistical comparison between the groups is shown by superscript letters, with different letters indicating $P < .05$ (n = 5-6 rats per group).

Lypolytic Activity

The lipolytic response of white adipocytes from olive oil–fed rats to either epinephrine, isoproterenol, BRL-37344, or CGP-12177 was always lower in pregnant versus virgin animals (Fig 1). The hyperinsulinemic condition produced by the hyperinsulinemic-euglycemic clamp substantially decreased the response to any of these agonists in adipocytes from virgin rats, although it did not modify the already low response detected in pregnant rats. These effects cause the dose-response curves for the different agents to appear similar for virgin and pregnant rats (Fig 1).

In white adipocytes from fish oil–fed rats, the response to epinephrine, isoproterenol, BRL-37344, or CGP-12177 was also lower in pregnant versus virgin rats (Fig 2). However, and in contrast to the effect of the hyperinsulinemic-euglycemic clamp in olive oil–fed virgin rats, in rats fed the fish oil diet, the hyperinsulinemic-euglycemic clamp did not modify the dose-response curve to any of the lipolytic agents in either virgin or pregnant rats, and values in virgin rats under the hyperinsulinemic-euglycemic condition remained higher than those in pregnant rats (Fig 2).

Lipolytic Potency of the Agonists

EC_{50} values were calculated as an index of the lipolytic potency for each β_3-AR agonist. With a few exceptions, the

Table 5. Effect of Olive Oil and Fish Oil Diets on Plasma Components in Virgin and 20-Day Pregnant Rats

	Olive Oil Diet		Fish Oil Diet	
Parameter	Virgin (n = 6)	Pregnant (n = 5)	Virgin (n = 6)	Pregnant (n = 6)
Glucose (mmol/L)	8.49 ± 0.23[a]	5.09 ± 0.16[b]	7.78 ± 0.39[a]	4.28 ± 0.23[b]
Insulin (pmol/L)	235 ± 41[a]	466 ± 70[b]	253 ± 26[a]	480 ± 38[b]
FFA (μmol/L)	464 ± 21[a]	856 ± 116[b]	329 ± 25[a]	901 ± 170[b]
Triglycerides (mmol/L)	0.96 ± 0.17[a]	1.71 ± 0.09[b]	0.64 ± 0.10[a]	1.73 ± 0.15[b]

NOTE. Results are the mean ± SE. Statistical differences between groups are shown by superscript letters, different letters indicate significant differences ($P < .01$).

Table 6. Phospholipid Fatty Acid Composition of Lumbar Fat Pad From 20-Day Pregnant and Virgin Rats Fed Olive Oil or Fish Oil Diets (g/100 g fatty acids)

	Olive Oil		Fish Oil	
Fatty Acid	Virgin (n = 6)	Pregnant (n = 5)	Virgin (n = 4)	Pregnant (n = 5)
12:0	0.01 ± 0.01	0.40 ± 0.25	0.01 ± 0.01	0.01 ± 0.01
14:0	1.37 ± 0.31	1.18 ± 0.48	1.74 ± 0.66	2.14 ± 0.33
15:0	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.43 ± 0.42
16:0	28.5 ± 1.2	26.0 ± 1.2	30.0 ± 2.2	29.9 ± 2.7
18:0	17.1 ± 1.3	16.2 ± 1.2	17.8 ± 1.6	19.1 ± 1.5
16:1 (n-7)	2.81 ± 0.5	3.71 ± 0.35	3.79 ± 0.82	2.81 ± 0.35
18:1 (n-9)	29.4 ± 1.3[a]	29.8 ± 1.8[a]	20.2 ± 0.4[a]	19.0 ± 1.3[b]
20:1 (n-9)	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.76 ± 0.50
22:1 (n-9)	0.01 ± 0.01	1.09 ± 0.48	0.01 ± 0.01	0.34 ± 0.23
18:2 (n-6)	10.8 ± 2.1[ab]	15.4 ± 3.4[a]	8.16 ± 1.06[ab]	5.58 ± 0.29[b]
18:3 (n-3)	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.83 ± 0.82
20:4 (n-6)	2.52 ± 1.59	3.51 ± 1.53	4.12 ± 2.27	1.62 ± 0.62
20:5 (n-3)	5.30 ± 0.69[a]	1.66 ± 0.43[a]	7.71 ± 1.14[b]	15.89 ± 0.76[c]
22:6 (n-3)	0.34 ± 0.34[a]	0.01 ± 0.01[a]	5.48 ± 1.34[b]	3.54 ± 0.63[b]
Total saturated	47.8 ± 1.6	43.8 ± 1.9	49.6 ± 1.9	51.6 ± 2.8
Total monounsaturated	32.2 ± 1.7[a]	34.6 ± 2.16[a]	25.0 ± 1.0[b]	23.1 ± 1.1[b]
Total polyunsaturated	16.6 ± 3.0[a]	21.3 ± 2.2[ab]	25.5 ± 2.6[ab]	27.9 ± 0.9[b]
Total (n-6)	13.8 ± 2.7[ab]	18.9 ± 2.1[a]	12.3 ± 3.1[ab]	7.20 ± 0.66[b]
Total (n-3)	5.60 ± 0.79[a]	1.66 ± 0.43[a]	13.2 ± 2.1[b]	20.5 ± 0.9[b]
(n-6)/(n-3)	2.38 ± 1.19[a]	10.6 ± 3.3[b]	1.08 ± 0.41[a]	0.37 ± 0.05[a]
Monounsaturated + polyunsaturated)/saturated ratio	1.08 ± 0.13[ab]	1.38 ± 0.08[a]	1.03 ± 0.07[b]	1.05 ± 0.08[ab]

NOTE. Results are the mean ± SE. Statistical differences between groups are shown by superscript letters; different letters for the same fatty acid indicate significant differences between the groups ($P < .05$).

lipolytic potency was highest (lowest EC_{50}) for BRL-37344, followed by isoproterenol and both CGP-12177 and epinephrine with values in the same range (Table 8). The lipolytic potency of each agonist was similar in adipocytes of untreated pregnant and virgin rats fed either diet. However, in adipocytes from rats under the hyperinsulinemic-euglycemic clamp condition, the lipolytic potencies of epinephrine and isoproterenol were significantly higher in tissues from pregnant rats fed olive oil. Also, the lipolytic potencies of isoproterenol, BRL-37344, and CGP-12177 were significantly higher in pregnant rats fed the fish oil diet. In fact, although the wide dispersion of EC_{50} data impedes a fine statistical comparison between the groups, it can be drawn from Table 8 that despite the similarities in the lipolytic potency for each agonist in adipocytes from untreated pregnant and virgin rats, there was an increase in the lipolytic potency for most of the agonists when tissues were from pregnant rats under the hyperinsulinemic-euglycemic condition as compared with virgin rats studied under the same conditions.

DISCUSSION

The present study shows that the maximal lipolytic responsiveness of β_3-AR agonists by adipocytes is impaired in cells from hyperinsulinemic rats fed a diet with 5% olive oil as a fat source. The effect appeared similar when using both nonselective β-AR agonists such as epinephrine and isoproterenol and β_3-AR selective agonists such as BRL-37344 and CGP-12177, the latest known to stimulate the β_3-AR exclusively,[33] indicating that it corresponds to a decreased β_3-adrenergic responsiveness. Rats were hyperinsulinemic either because they were pregnant or because they were studied under a hyperinsulinemic-euglycemic clamp condition either pregnant or virgin. The olive oil diet has a fatty acid composition that allows a balanced fatty acid composition of adipocyte phospholipids, as shown by a monounsaturated plus polyunsaturated to saturated fatty acid ratio similar to that found by others testing higher proportions of fat but in a mixture of vegetable and animal oils.[24] The present

Table 7. Circulating Glucose and Insulin, and the Insulin Sensitivity Index in Rats Studied Under the Hyperinsulinemic-Euglycemic Clamp Condition

	Olive Oil Diet		Fish Oil Diet	
Parameter	Virgin (n = 6)	Pregnant (n = 8)	Virgin (n = 6)	Pregnant (n = 7)
Blood glucose (mmol/L)	8.1 ± 0.5[a]	5.1 ± 0.2[b]	7.7 ± 0.4[a]	4.9 ± 0.2[b]
Plasma insulin (pmol/L)	1,281 ± 323	1,785 ± 472	951 ± 333	2,052 ± 437
Glucose disposal rate (μmol/min/kg)	274 ± 23[a]	142 ± 17[b]	332 ± 21[a]	172 ± 12[b]
Insulin sensitivity index (10^{-4} dL · min^{-1} · kg^{-1} · μU^{-1} mL)	28.5 ± 7.0[a]	13.0 ± 2.6[b]	44.6 ± 11.0[a]	11.8 ± 1.1[b]

NOTE. Results are the mean ± SE. Statistical differences between groups are shown by superscript letters; different letters for the same variable indicate significant differences ($P < .05$).

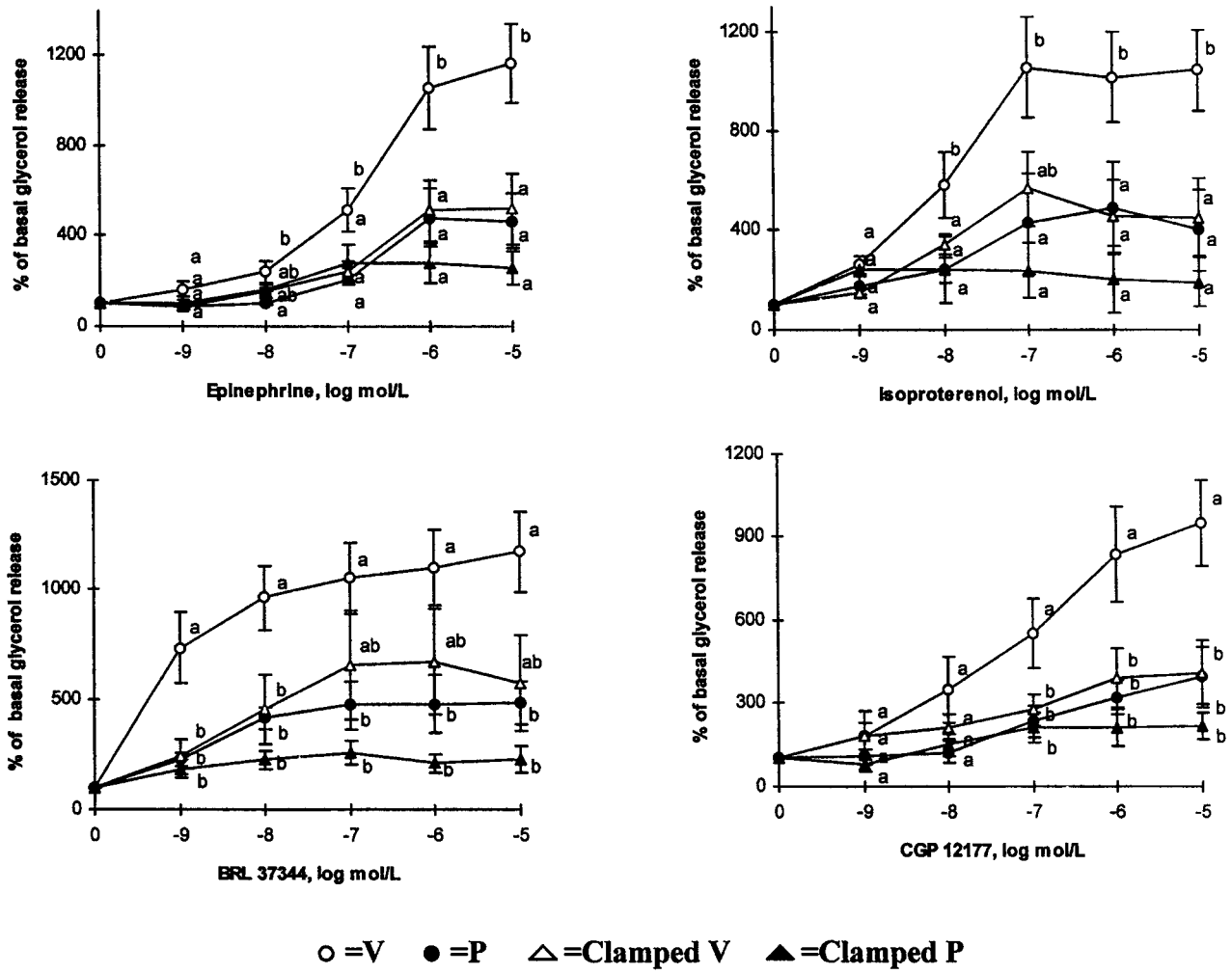

Fig 1. Dose-response curves (mean ± SE) for lipolysis stimulated by epinephrine, isoproterenol, BRL-37344, and CGP-12177 in adipocytes from 20-day pregnant rats (P, n = 5-6) or virgin rats (V, n = 6-8) fed a diet containing 5% olive oil and receiving no treatment, or at 60 minutes of a hyperinsulinemic-euglycemic clamp (0.4 U insulin/kg/h). Isolated fat cells from lumbar adipose tissue were incubated with the indicated agent, and glycerol release to the medium was measured and used as a lipolytic index. The curves are plotted as a percentage of the basal rate (incubations in the absence of any agent). Statistical comparison between the points for each agonist concentration is shown (different letters indicate significant differences between the points, $P < .05$).

findings agree with those previously reported on the effect of insulin exposure to inhibit the lipolytic response to β-adrenergic agonists in either cultured 3T3L1 cells[35] or isolated human[18] or rat[17] adipocytes, constituting a physiologic base for the role of hyperinsulinemia in decreasing β_3-AR activity in adipose tissue. In the case of pregnancy, hyperinsulinemia was accompanied by an insulin-resistant condition, as shown by the decreased glucose disposal rate and the insulin sensitivity index in the hyperinsulinemic-euglycemic clamp; these results agree with previous findings in rats receiving a standard pellet diet.[10,36] Other conditions of hyperinsulinemia and insulin resistance, as reported in the insulin resistance (metabolic) syndrome in humans, have also been shown to present a lipolytic resistance to catecholamines.[20] Besides, a decrease in the maximal lipolytic effect of isoproterenol has been reported in pregnant ewes.[37] All of the above findings support the idea that despite the insulin-resistant condition of the mother during late pregnancy, her hyperinsulinemia is responsible for a decreased β_3-AR responsiveness.

The 5% fish oil diet used in the present study was not sufficient to decrease the fat pad weight in virgin and pregnant rats, although values remained higher in the latter, probably due to the well-known tendency for fat depot accumulation during pregnancy.[5,38] An effect of the fish oil diet to decrease fat depots, corresponding to a decrease in adipocyte size, has been described,[39-42] but with much higher doses (20%), and such a difference in size could affect β-adrenergic responsiveness due to differences in receptor density.[43] Besides the lack of effect of the 5% fish oil diet on fat pad weight, lipolytic values were expressed per unit of lipids in the preparation and were corrected as a percentage of values obtained in the absence of any agent, minimizing potential intergroup differences.

The 5% fish oil diet used here also caused an enrichment in n-3 polyunsaturated fatty acids in the composition of adipose

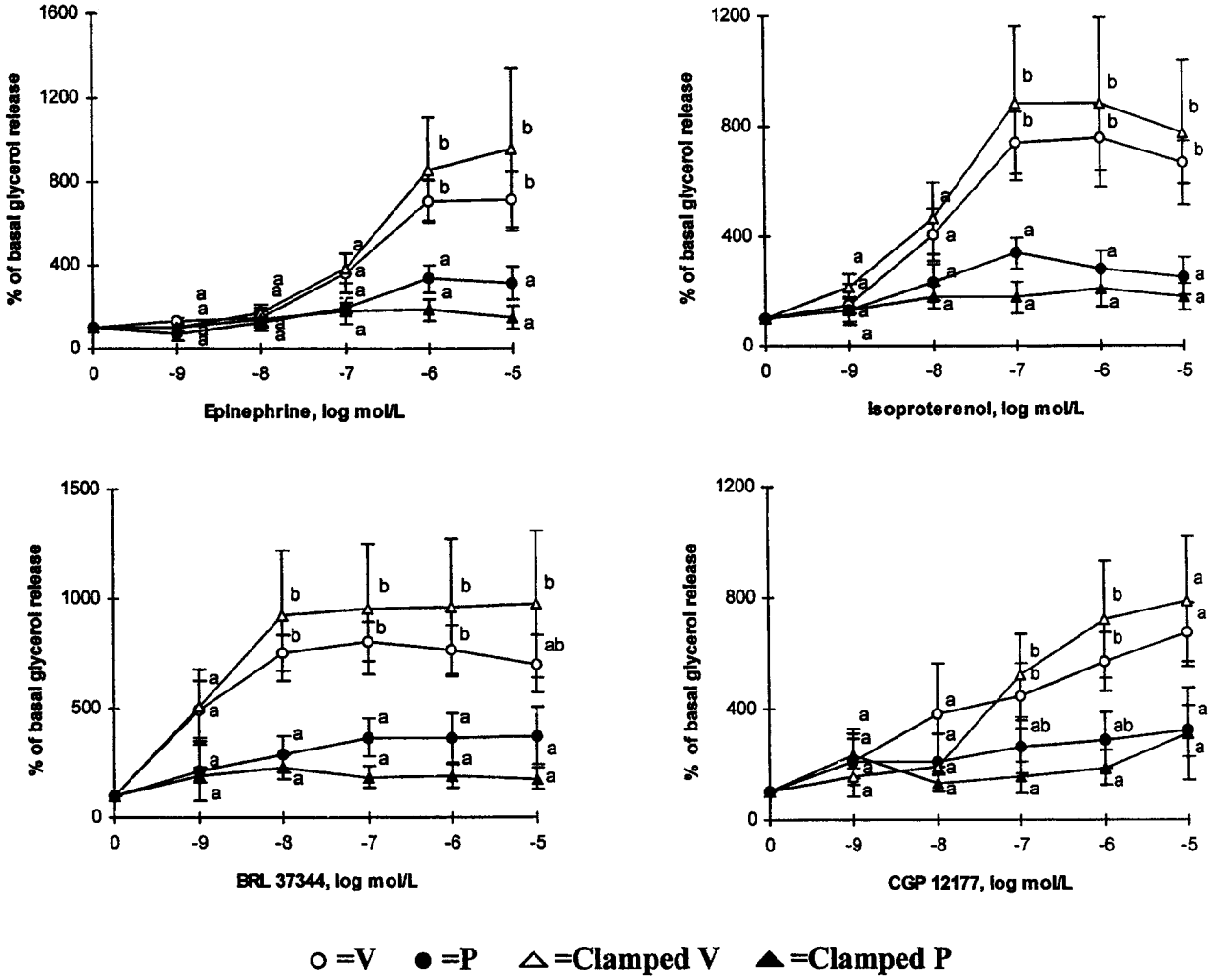

Fig 2. Dose-response curves (mean ± SE) for lipolysis stimulated by epinephrine, isoproterenol, BRL-37344, and CGP-12177 in adipocytes from 20-day pregnant rats (P, n = 5-6) or virgin rats (V, n = 6-8) fed a diet containing 5% fish oil and receiving no treatment, or at 60 minutes of a hyperinsulinemic-euglycemic clamp (0.4 U insulin/kg/h). Isolated fat cells from lumbar adipose tissue were incubated with the indicated agent, and glycerol release to the medium was measured and used as a lipolytic index. The curves are plotted as a percentage of the basal rate (incubations in the absence of any agent). Statistical comparison between the points for each agonist concentration is shown (different letters indicate significant differences between the points, $P < .05$).

tissue fatty acid phospholipids, although the proportional difference versus the values in rats fed the olive oil diet was smaller than that found for these same fatty acids in the diets. This finding agrees with data reported by others,[24,42,44] supporting the notion that these fatty acids are not stored in adipose tissue in similar proportion to their composition in the diet, because they are preferentially oxidized rather than stored. This study shows that the effect of a hyperinsulinemic-euglycemic clamp to reduce β_3-adrenergic agonist responsiveness in virgin rats disappeared when rats were fed the fish oil diet, whereas the effect of pregnancy was not affected by this diet. The present findings do not explain this difference, but it is known that dietary fish oil at a much higher proportion (30%) than the one used in the present study (5%) may improve adipocyte insulin action.[24] Therefore, it appears that a certain dietary change able to modify adipocyte phospholipid composition may also modify the sensitivity of the tissue to insulin in relation to its β_3-AR agonistic responsiveness. The mechanism underlying the decreased effect to hyperinsulinemia in adipose tissue of virgin rats fed the fish oil diet may well reside in their enhanced proportion of polyunsaturated fatty acids within the phospholipid bilayer, since this effect is known to determine the physicochemical properties of membranes that in turn influence cellular functions, including receptor activity and hormone responsiveness.[45,46]

The fish oil diet used in the present study was unable to modify the insulin-resistant condition detected in pregnant rats. The reported effects of dietary fish oil for enhancing insulin sensitivity in rats made insulin-resistant by either high-fat or sucrose and fat feeding are controversial, from studies that showed significant changes,[24,47] to those that found in vivo insulin-stimulated glucose metabolism without an effect on white adipose tissue responsiveness,[48] and those that were unable to modify the insulin resistance.[49] The different response

Table 8. EC_{50} for Epinephrine, Isoproterenol, BRL-37344, and CGP-12177 on Adipocytes From Virgin and Pregnant Rats Fed an Olive Oil or Fish Oil Diet Under Untreated or Hyperinsulinemic-Euglycemic Clamp (0.4 U insulin/kg/h) Conditions

	Untreated		Hyperinsulinemic-Euglycemic Clamp	
Parameter	Virgin	Pregnant	Virgin	Pregnant
Olive oil diet				
Epinephrine	201 ± 50[a]	125 ± 38[a]	209 ± 61[a]	10 ± 5[b]
Isoproterenol	24 ± 9[a]	32 ± 19[a]	11 ± 3[a]	0.5 ± 0.1[b]
BRL-37344	4 ± 2[a]	4 ± 2[a]	7 ± 3[a]	5 ± 3[a]
CGP-12177	131 ± 73[a]	177 ± 62[a]	64 ± 33[a]	44 ± 21[a]
Fish oil diet				
Epinephrine	168 ± 76[a]	119 ± 71[a]	95 ± 29[a]	20 ± 15[a]
Isoproterenol	14 ± 6[a]	13 ± 6[a]	5.0 ± 0.4[a]	1.2 ± 0.6[b]
BRL-37344	2 ± 1[a]	4 ± 2[a]	1.3 ± 0.3[a]	0.5 ± 0.1[b]
CGP-12177	177 ± 89[a]	214 ± 95[a]	67 ± 16[a]	0.5 ± 0.2[b]

NOTE. Results are the mean ± SE. Lipolytic potencies (EC_{50}, nmol/L) were calculated by computer-assisted fitting of the concentration-response curves presented in Figs 1 and 2 (n = 5-6 experiments per group). Statistical differences within the same agent and condition are shown by superscripts (different letters indicate significant differences, $P < .05$).

seems to depend not only on the insulin-resistant model used but, more importantly, also on the percentage of fish oil in the diet, and the amount (5%) used in the present study was the lowest compared with other reports. Previous reports using fish oil doses over 5% have demonstrated a decrease in plasma FFA or triglyceride levels,[50,51] variables not affected in the present study. However, these variables themselves could increase the insulin action induced by fish oil, as suggested by Storlien et al.[48]

The present study does not allow us to determine whether hormonal factors other than insulin which are important during pregnancy (eg, placental hormones) may also contribute to the decreased response to β-adrenergic agonists, and more direct experiments would be needed to clarify this point. However, the highest lipolytic potency together with the lowest maximal responsiveness for most of the agents studied under conditions of pregnancy and a hyperinsulinemic-euglycemic clamp, a condition wherein the highest insulin level is attained as reported here, would support our hypothesis that insulin plays a key role in the decreased lipolytic β_3-adrenergic response in adipocytes during pregnancy.

We may therefore conclude that hyperinsulinemic conditions, like those caused by pregnancy or the hyperinsulinemic-euglycemic clamp, reduce β_3-AR lipolytic responsiveness, and such a response depends on the fat composition of the diet, which modifies the adipocyte phospholipid fatty acid composition even though it does not affect in vivo insulin sensitivity.

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