Am J Physiol Endocrinol Metab 286: E488–E494, 2004.
First published November 4, 2003; 10.1152/ajpendo.00199.2003.

Additive effects of cortisol and growth hormone on regional and systemic lipolysis in humans

C. B. Djurhuus,[1] C. H. Gravholt,[1] S. Nielsen,[2] S. B. Pedersen,[4] N. Møller,[1,2] and O. Schmitz[1,3]

[1]*Department of Endocrinology and Diabetes, University Hospital of Aarhus, Aarhus Kommunehospital;* [2]*Institute of Experimental Clinical Research, and* [3]*Institute of Clinical Pharmacology, University of Aarhus; and* [4]*Department of Endocrinology and Metabolism, University Hospital of Aarhus, Aarhus Amtssygehus DK-8000 Aarhus, Denmark*

Submitted 2 May 2003; accepted in final form 13 October 2003

Djurhuus, C. B., C. H. Gravholt, S. Nielsen, S. B. Pedersen, N. Møller, and O. Schmitz. Additive effects of cortisol and growth hormone on regional and systemic lipolysis in humans. *Am J Physiol Endocrinol Metab* 286: E488–E494, 2004. First published November 4, 2003; 10.1152/ajpendo.00199.2003.—Growth hormone (GH) and cortisol are important to ensure energy supplies during fasting and stress. In vitro experiments have raised the question whether GH and cortisol mutually potentiate lipolysis. In the present study, combined in vivo effects of GH and cortisol on adipose and muscle tissue were explored. Seven lean males were examined four times over 510 min. Microdialysis catheters were inserted in the vastus lateralis muscle and in the subcutaneous adipose tissue of the thigh and abdomen. A pancreatic-pituitary clamp was maintained with somatostatin infusion and replacement of GH, insulin, and glucagon at baseline levels. At $t = 150$ min, administration was performed of NaCl (I), a 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ hydrocortisone infusion (II), a 200-μg bolus of GH (III), or a combination of II and III (IV). Systemic free fatty acid (FFA) turnover was estimated by [9,10-^{3}H]palmitate appearance. Circulating levels of glucose, insulin, and glucagon were comparable in I-IV. GH levels were similar in I and II (0.50 $\pm$ 0.08 μg/l, mean $\pm$ SE). Peak levels during III and IV were ~9 μg/l. Cortisol levels rose to ~900 nmol/l in II and IV. Systemic (i.e., palmitate fluxes, s-FFA, s-glycerol) and regional (interstitial adipose tissue and skeletal muscle) markers of lipolysis increased in response to both II and III. In IV, they were higher and equal to the isolated additive effects of the two hormones. In conclusion, we find that GH and cortisol stimulate systemic and regional lipolysis independently and in an additive manner when coadministered. On the basis of previous studies, we speculate that the mode of action is mediated though different pathways.

microdialysis; corticosteroids

ADIPOSE TISSUE CONSTITUTES the largest fuel reservoir in the body, vastly exceeding available glycogen and protein stores (5). It has been estimated that the fat depots provide energy for ~2 mo of living in lean subjects compared with the energy stored as glycogen, lasting one day (37). Free fatty acids (FFA) from triacylglycerol (TAG) breakdown are a major energy source for heart and skeletal muscle (40).

Regulation of FFA release from adipocytes involves the enzyme hormone-sensitive lipase (HSL) (16, 21). Upon activation, HSL is translocated to the periphery of the intracellular fat droplet (4). There, the enzyme hydrolyzes TAG to FFA and glycerol. Studies on postabsorptive skeletal muscle fuel metabolism have until recently focused primarily on carbohydrates or FFA derived from the adipose tissue as sources of energy. However, it has become increasingly clear that TAG located in the muscle contributes as well, especially at rest and during moderate exercise (25, 42).

Both growth hormone (GH) and cortisol, among other effects, ensure substrate supplies, postabsorptively, during prolonged fasting (3, 33) and stress (43). Previous studies have demonstrated isolated effects of physiological levels of GH on lipolysis (17, 32, 34), and recently we (10) have demonstrated acute lipolytic effects of cortisol as assessed by interstitial glycerol levels, serum FFA, and glycerol and systemic FFA turnover.

In vitro studies have revealed lipolytic actions of GH and an inhibitory effect of glucocorticoids, whereas a combined effect of GH and cortisol exceeding the GH-induced lipolysis has been described (36). Another in vitro study found glycerol release to be increased with isolated and concomitant stimulation of lipolysis with GH and dexamethasone (DEX) (44). The overall explanation for this finding could be that GH in the presence of DEX downregulated the G-coupled α_2-receptors and, hence, increased lipolysis.

Because GH and cortisol are cosecreted during stress conditions, it is conceivable that both hormones are involved in the regulation of adipose tissue metabolism during fasting and stress. The present study was undertaken to gain further insight into the isolated and combined impact of GH and cortisol on overall and interstitial subcutaneous adipose tissue and skeletal muscle TAG metabolism by applying systemic tracer dilution and regional microdialysis techniques and utilizing glycerol as a marker of interstitial lipolysis (13, 19).

MATERIALS AND METHODS

Before the study, a power analysis was performed on the basis of findings by Divertie et al. (9) and Gravholt et al. (17). On that basis we included seven healthy young male subjects [26.6 $\pm$ 0.2 yr and body mass index 22.9 $\pm$ 0.5 kg/m^2 (mean $\pm$ SE)]. All gave their informed consent after receiving oral and written information according to the Declaration of Helsinki II. The study was approved by the Scientific Ethics Comittee of Aarhus County.

Experimental protocol. The study was carried out as a single-blinded, placebo-controlled, randomized trial.

The subjects were admitted to the research laboratory at 0700 ($t = -150$ min) after an overnight fast (~10 h). The participants were instructed not to perform any physical exercise or ingest alcohol 1 day before the start of the study and to consume a weight-maintaining, carbohydrate-rich diet for 3 days before examination.

Address for reprint requests and other correspondence: C. B. Djurhuus, Dept. of Endocrinology M, Aarhus Kommunehospital, Aarhus Univ. Hospital, DK-8000 Aarhus C, Denmark (E-mail: cbd@dadlnet.dk).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

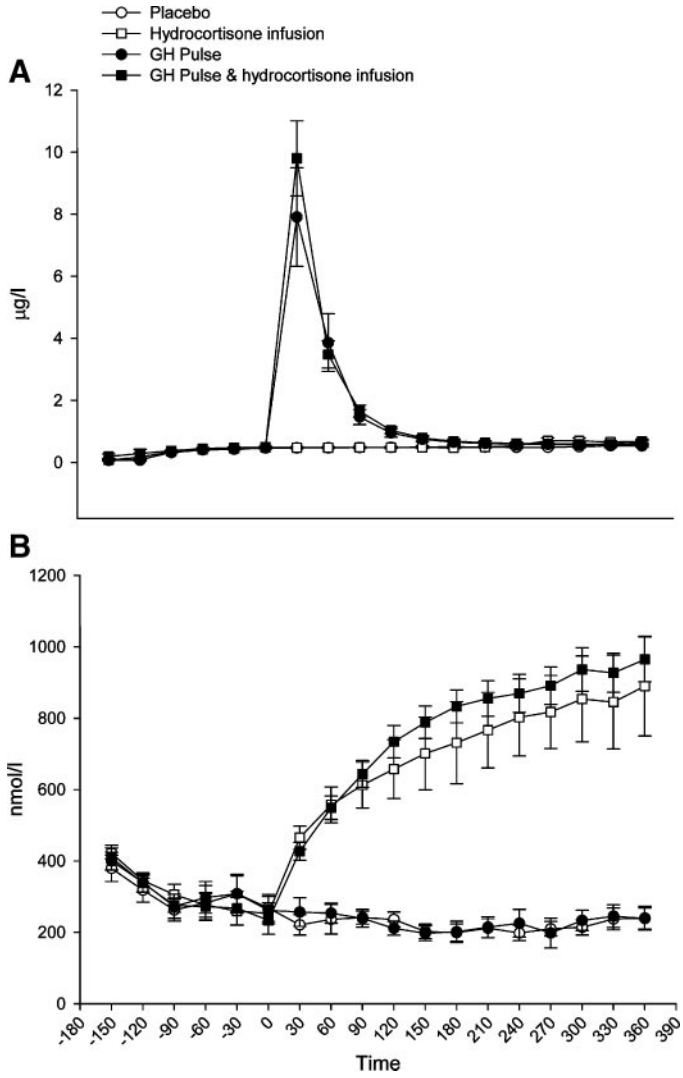

Fig. 1. Serum concentration of administered hormones. *A*: growth hormone (GH). *B*: cortisol. Symbols denote the 4 treatment groups: placebo, I; hydrocortisone, II; GH pulse, III; GH + hydrocortisone, IV. Time, in min.

Upon arrival at the research laboratory, subjects were placed in the supine position in bed wearing light hospital clothing in a room with an ambient temperature of 22–24°C, and they remained in that position throughout the study. Two intravenous catheters (Venflon; Viggo, Helsingborg, Sweden) were inserted in the antecubital vein of the left arm and in a dorsal vein of the left hand. The latter was placed in a heated box at 65°C, allowing for arterialized blood samples to be drawn (1). Blood samples were drawn every 30 min and analyzed for plasma glucose (PG), FFA, glycerol, cortisol, GH, glucagon, insulin, C-peptide, and catecholamines.

At $t = -120$ min, infusion of somatostatin (330 μg/h; Ferring Pharmceuticals), insulin (0.08 mU·kg^{-1}·min^{-1} Actrapid; Novo Nordisk, Bagsvaerd, Denmark), and GH (2 ng·kg^{-1}·min^{-1} Norditropin; Novo Nordisk) was commenced. Infusion of glucagon (0.5 ng·kg^{-1}·min^{-1} Glucagen; Novo Nordisk) was not added until $t = -90$ min.

Euglycemia was intended and isotonic glucose was infused to ensure PG >80 mg/dl to prevent hypoglycemia-induced breakthrough of the pancreatic clamp.

At $t = 0$ min, an infusion in a randomized manner of either 0.9% NaCl (I), infusion of hydrocortisone sodium succinate 2 μg·kg^{-1}·min^{-1} (Solu-Cortef, Phamacia Upjohn; II), a bolus of 200 μg GH (Norditropin; III), or a combined bolus of GH and hydrocortisone succinate infusion (IV) was initiated. Infusions were continued throughout the duration of the study ($t = 360$ min).

Microdialysis. After application of a local analgesic, 0.25 ml of lidocaine, a microdialysis catheter (CMA-60; CMA, Stockholm, Sweden) was inserted at $t = -150$ min in the subcutaneous adipose tissue ~5 cm dextrolateral of the umbilicus and in the right femoral subcutaneous adipose tissue. Lidocaine (1 ml) was injected superficial to the fascia of the lateral vastus muscle 10 and 14 cm above the patella before insertion. Correct placement of the microdialysis catheters in the muscle was confirmed by the presence of muscle twitches during insertion. Two catheters were used to ensure survival of at least one, as muscle twitches can disrupt the microdialysis membrane. The microdialysis catheters have a molecular cut-off of 20 kDa and a membrane length of 30 mm.

Before insertion, the catheters were manually flushed with perfusion fluid (Ringer Chloride T1, CMA: 147 mmol/l Na^+, 1.4 mmol/l K^+, 2.3 mmol/l Ca^{2+}, 156 mmol/l Cl^-, pH 6, osmolality 290 mosmol/kg) to allow for clearance of air bubbles from the microdialysis membranes. Additionally, dipping of the membranes in the perfusate medium was performed as recommended by CMA. The microdialysis systems were perfused at a flow rate of 1 μl/min using the CMA-107 perfusion pump (CMA). The relative recovery of interstitial glycerol was assessed by the internal reference method with [^{3}H]glycerol (28, 41).

[^{3}H]glycerol was added to the perfusate to obtain ~1,000 cpm/μl. Perfusate and dialysate were counted using a Wallac 1450 liquid scintillation counter applying the Optiphase supermix scintillation fluid. Changes in interstitial glycerol concentration can be seen as an index of lipolysis (2, 19, 22).

Sampling of the interstitial fluid commenced at $t = -90$ min, allowing for 60 min of equilibration to minimize the influence of local edema and hemorrhage. The sampling was performed every 30 min and continued until $t = 360$ min.

Palmitate turnover. Systemic palmitate fluxes were measured at $t = 240$–270 min by use of the isotope dilution technique and steady-state equations. [9,10-^{3}H]palmitate (Laegemiddelstyrelsen, Copenhagen, Denmark) was infused continuously at 0.3 μCi/min from 210 to 270 min, and blood samples were drawn for analysis of palmitate concentration and specific activity (SA). Steady state of SA was verified ($t = 240$, 255, and 270 min) for each individual.

Table 1. *Time-averaged values during baseline and stimulation*

Hormones	I Placebo	II Hydrocortisone	III GH Pulse	IV GH Pulse + Hydrocortisone
Insulin, pmol/l				
Baseline	37[19;82]	34[13;69]	36[29;91]	30[16;82]
Stimulated	33[21;69]	33[27;71]	38[30;75]	31[24;70]
C-peptide, pmol/l				
Baseline	332[56;1,042]	223[20;592]	417[98;1,082]	231[72;671]
Stimulated	56[0;342]	10[0;97]	60[0;350]*	50[0;157]
Glucagon, ng/l				
Baseline	16[4;78]	22[6;88]	18[4;64]	20[1;88]
Stimulated	18[7;54]	20[4;36]	19[2;39]	15[6;44]
Epinephrine, pg/ml				
Baseline	68[50;170]	51[50;190]	50[25;392]	50[50;469]
Stimulated	50[50;210]	50[50;208]	53[31;340]	54[50;146]
Norepinephrine, pg/ml				
Baseline	363±79	292±58	303±46	323±78
Stimulated	305±47	284±63	300±37	283±43

Nonparametric data are expressed as median[min;max]; parametric data are means ± SE. GH, growth hormone. Baseline, $t = -120$–0 min; stimulation, $t = 0$–360 min [analysis of area under curve (AUC) during baseline and stimulation]. I–IV, treatments (see *Experimental protocol* in text.) *$P < 0.05$.

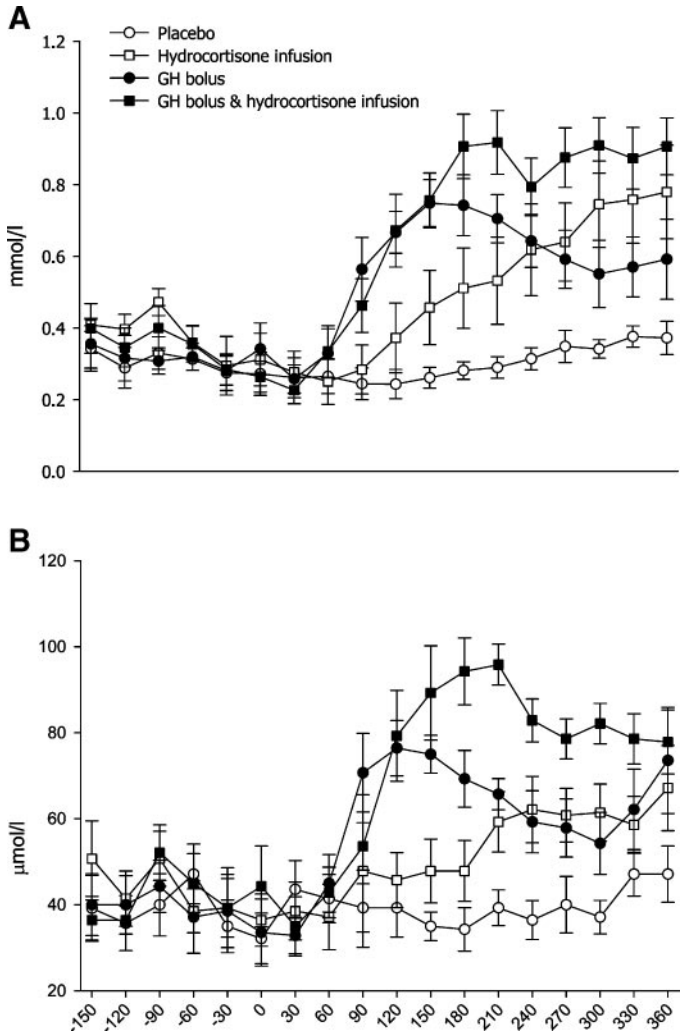

Fig. 2. *A*: serum concentration free fatty acids (FFA) during exposures. II > I,* III > I,* IV > III and II,* and IV > I*. *B*: serum glycerol during exposures. II > I ($P = 0.07$), III > I,* IV > III and II,* and IV > I*. *$P < 0.05$.

Plasma palmitate concentration and SA were determined by HPLC (31) using [$^2H_{31}$]palmitate as internal standard (24). Systemic palmitate flux (μmol/min) was calculated using the [9,10-^{3}H]palmitate infusion rate (dpm/min) divided by the steady-state palmitate SA (dpm/μmol).

The bolus administration of GH induces a non-steady-state appearance of FFA. To account for this, we analyzed FFA and detected stable FFA concentrations at 240–270 min. Further analysis of palmitate SAs confirmed that palmitate SA was stable throughout this period during the four treatments (see Fig. 4, *inset*). Linear regression showed that the slope of the curves did not differ from zero in any of the treatments (I, $P = 0.86$; II, $P = 0.37$; III, $P = 0.71$; and IV, $P = 0.38$). Likewise, no difference was observed between treatments ($P = 0.30$). On this basis, we applied steady-state equations for subsequent calculation of palmitate fluxes.

Skeletal muscle blood flow. Skeletal muscle blood flow (SMBF) was assessed by plethysmography applied to the thigh contralateral to the microdialysis probes. A cuff was inflated to 300 mmHg at the level of the patella to measure flow exclusively in the thigh. Although this method includes skin and bone as well as muscle tissue, the majority of the tissue perfused is skeletal muscle. Measurements were performed at $t = 0$, 120, 240, and 360 min in duplicate.

Adipose tissue blood flow. We have previously assessed the impact of similar GH and cortisol exposures on adipose tissue blood flow (ATBF) with the xenon washout technique (10, 17). Here, we did not detect any changes in ATBF after GH or cortisol administration. We therefore assume that coadministration of GH and cortisol does not influence ATBF.

Assays. Plasma glucose was analyzed in duplicate using the glucose oxidase method (Beckman Coulter, Palo Alto, CA). Measurements were performed immediately to determine the isotonic glucose infusion rate.

Serum GH was analyzed with a double monoclonal immunofluorometric assay (Delfia, Wallac Oy, Turku, Finland). Serum C-peptide and insulin were measured with an immunoassay (DAKO, Glostrup, Denmark). Plasma glucagon was measured by an in-house radioimmunoassay modified from that in Ref. 35. Serum cortisol was measured with a solid-phase, time-resolved fluoroimmunoassay (Delfia). Serum FFA was determined by a colorimetric method employing a commercial kit (Wako Pure Chemical Industries, Neuss, Germany). Blood levels of glycerol were assayed with an automated fluorometric method (27). Epinephrine and norepinephrine were measured by HPLC (6). Glycerol in the microdialysis dialysate was measured in duplicate by an automated spectrophotometric kinetic enzymatic analyzer (CMA-600).

Statistical analysis. Results are expressed as time-averaged values during baseline ($t = -150$ to 0) and the stimulated period ($t = 0$–360 min). Statistical difference is based on area under the curve $(AUC)_{0–360\ min}$. Because of the difference in the administration of GH (bolus) and hydrocortisone succinate (infusion), subanalysis based on AUC was performed in the intervals 0–150 and 240–360 min where stated. AUC was calculated using ICUPilot version 1.0 (CMA) using the trapezoid method. Statistical analysis was performed using SPSS for Windows version 11.0 (SPSS, Chicago, IL). Normality of the data was tested with the Kolmogorov-Smirnov test of normal distribution. Where $P > 0.20$, the data were considered to be normally distributed.

Equality among the four treatments was assessed by repeated measures or Friedman's test for *k*-related samples where appropriate. Between-treatment differences were assessed by Student's *t*-test for

Table 2. *Time-averaged values within intervals*

Circulating Metabolites		I Placebo	II Hydrocortisone	III GH Pulse	IV GH Pulse + Hydrocortisone
FFA, mmol/l	$AUC_{-150–0\ min}$	0.310±0.05	0.386±0.05	0.316±0.05	0.357±0.06
	$AUC_{0–360\ min}$	0.300±0.04	0.519±0.10	0.580±0.08	0.719±0.08
		II > I*·III > I*·IV > I*·IV > II*·IV > III*			
Glycerol, μmol/l	$AUC_{-150–0\ min}$	39.4±6.9	44.1±6.7	40.0±7.4	41.9±6.1
	$AUC_{0–360\ min}$	40.0±5.3	52.9±8.1	61.2±7.0	74.2±6.7
		II > I ($P = 0.07$)·III > I*·IV > I*·IV > II*·IV > III*			
[9,10-^{3}H]palmitate, μmol/min	240–270 min	94.8±8.1	160.0±19.2	148.1±12.9	206.3±11.0
		II > I*·III > I*·IV > I*·IV > II*·IV > III*			

Analysis of AUC during baseline and stimulation. FFA, free fatty acids. *$P < 0.05$.

related samples or Wilcoxon signed rank test (for related samples). *P* values < 0.05 were considered significant.

Parametric data are presented as means ± SE and nonparametric data as medians [min;max].

RESULTS

Circulating hormones. Infusion of hydrocortisone succinate resulted in steady-state cortisol levels of II: 829 ± 118 vs. IV: 907 ± 56 nmol/l, [not significant (NS)]. During GH bolus infusion, GH levels rose to peak values of III: 7.91 ± 1.59 vs. IV: 9.80 ± 1.21 μg/l at $t = 30$ min (NS) (Fig. 1).

No differences were detected in circulating levels of insulin, glucagon, and C-peptide in I vs. II, III, or IV, with the exception of C-peptide (see Table 1) in III being higher (III: 60[0;350] pmol/l, $P < 0.05$, vs. I: 56[0;342], II: 10[0;97], and IV: 50[0;157] pmol/l). The insulin levels did not differ ($P = 0.48$).

Circulating metabolites. Serum FFA rose to the same magnitude and within the same time frame as seen previously with GH (17) and hydrocortisone (10), with a peak value of III: 0.748 ± 0.07 mmol/l after 150 min and II: 0.779 ± 0.131 mmol/l at $t = 360$ min (Fig. 2 and Table 2).

The combined administration of GH and hydrocortisone succinate resulted in a maximal value of 0.906 ± 0.08 mmol/l at $t = 360$ min, being higher than both II and III ($P < 0.05$).

Serum glycerol revealed corresponding changes.

Interstitial glycerol concentrations. Interstitial skeletal muscle glycerol (ISMG) showed changes mimicking the ones observed in serum (Fig. 3 and Table 3). There was no difference between the two insertion sites (10 and 14 cm above patella), and for this reason combined results are presented. At baseline, the ISMG was ~50% higher than serum glycerol (68 ± 6 vs. 42 ± 4 μmol/l, $P < 0.01$). During the entire duration of the experiment, ISMG declined to a level of 34 ± 4 μmol/l at $t = 360$ min. During hydrocortisone infusion (II), the decline was dampened significantly, with levels at $t = 360$ min of 50 ± 3 μmol/l ($P < 0.01$). GH administration resulted in a glycerol surge with peak values at $t = 150$ min of 63 ± 7 μmol/l, being significantly different from placebo in the interval 0–150 min ($P < 0.01$). The combined effect of GH and hydrocortisone (IV) resulted in peak levels at $t = 150$ min of 79 ± 9 μmol/l, whereas the plateau at $t = 360$ min was 64 ± 7 μmol/l, both being respectively different from III and II, when the intervals 0–150 min ($P < 0.01$) and 240–360 min ($P = 0.11$) were analyzed, although the latter did not reach statistical significance.

We did not observe any disruptions of the microdialysis membranes, probably because we studied resting muscle.

Results in interstitial subcutaneous adipose tissue measurements were blemished with an error when relative recovery was corrected for. When the uncorrected values were analyzed, similar changes were seen as in serum or interstitial muscle.

We tested the dialysate for autoscintillation, which was not found, and, since the uncorrected interstitial adipose tissue values were more trustworthy, we suspect that quenching introduced the error. Because of the limited amount of dialysate (~30 μl), we were not able to test another scintillation fluid. Because recovery increases over time (Fig. 3*A*), we present the uncorrected values corrected for mean adipose tissue recovery during the intervals −90–0, 0–150, and 240–360 min in Table 3.

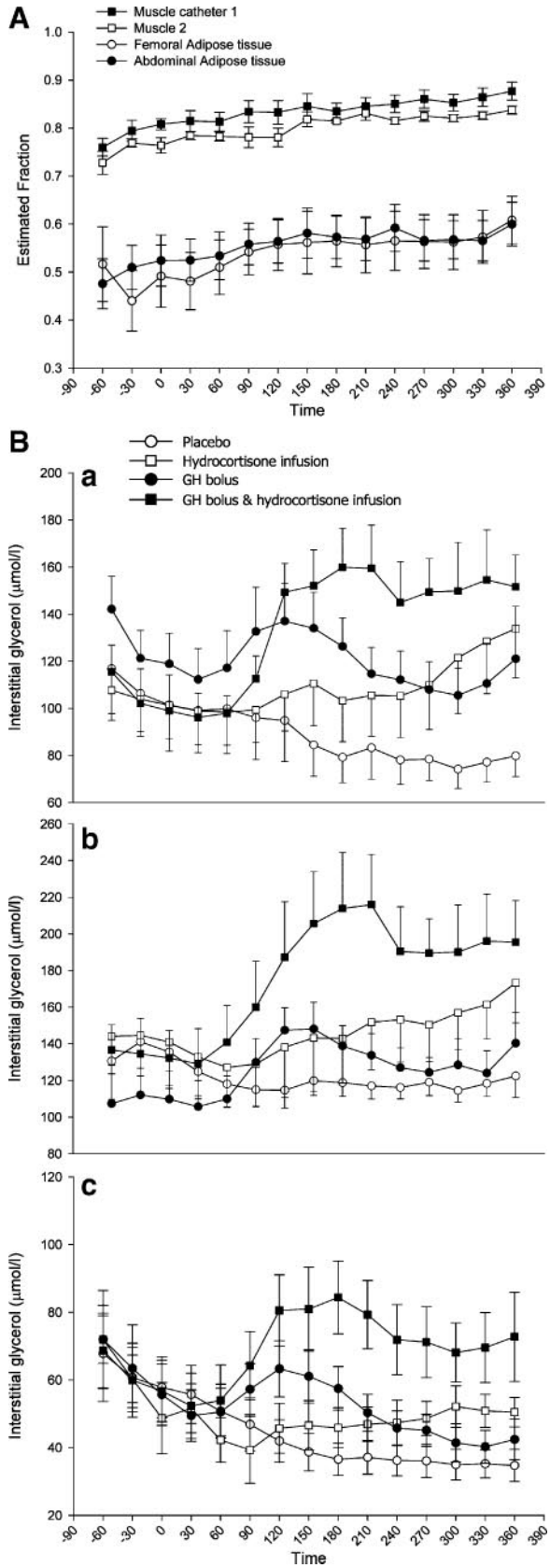

Fig. 3. *A*: relative recovery in skeletal muscle and subcutaneous adipose tissue assessed by internal reference method using [^{3}H]glycerol. *B*: interstitial glycerol in sc femoral adipose tissue (*a*); sc abdominal adipose tissue (*b*); and skeletal muscle (*c*). See text and Table 3 for statistics.

Table 3. *Time-averaged values during baseline and stimulation*

Interstitial Glycerol, μmol/l		I Placebo	II Hydrocortisone	III GH Pulse	IV GH Pulse + Hydrocortisone
Skeletal muscle	$AUC_{-90–0\ min}$	61±8	66±6	65±11	65±10
	$AUC_{0–150\ min}$	43±6	47±6	61±7*	71±9*
		III > I*·IV > I*·IV > II*·IV > III*			
	$AUC_{240–360\ min}$	35±4	50±3	47±6*	65±8*
		II > I*·III > I*·IV > I*·IV > II*·IV > III*			
sc Abdominal adipose tissue	$AUC_{-90–0\ min}$	237±31	214±48	230±32	278±30
	$AUC_{0–150\ min}$	197±17	191±47	255±24	313±41
		III > I (P = 0.06)·IV > I (P = 0.07)			
	$AUC_{240–360\ min}$	194±13	233±49*	231±22	363±41*
		II > I*·IV > I*·IV > III*			
sc Femoral adipose tissue	$AUC_{-90–0\ min}$	244±43	189±25	264±28	214±27
	$AUC_{0–150\ min}$	180±31	180±27	248±30	244±22
	$AUC_{240–360\ min}$	138±16	202±35*	197±26	265±31*
		II > I*·IV > I*·IV > III*			

Values are means ± SE. Baseline, t = −90–0 min; stimulation, t = 0–150 and 240–360 min (paired analysis of treatments). Analysis of interstitial glycerol uncorrected for recovery is performed for subcutaneous (sc) parameters. Average time-related recovery correction is made for the time-averaged values. *P < 0.05.

The relative recoveries, measured as $C_{perfusate} - C_{dialysate}/C_{perfusate}$ [C being counts per minute (cpm) for [2-^{3}H]glycerol], were comparable to the ones obtained previously with a flow rate of 1 μl/min (39) (Fig. 3*A*).

Palmitate metabolism. Palmitate fluxes increased due to both GH and hydrocortisone administration (P < 0.05 vs. placebo; Fig. 4). No difference was detected between GH and hydrocortisone fluxes, and both fluxes were significantly lower than the palmitate fluxes observed in the combined administration of GH (P < 0.01) and hydrocortisone (P < 0.05).

The interindividual coefficients of variation (CV) of SA during this interval were I, 9.2; II, 8.0; III, 6.3; and IV, 5.8%. No difference was found between them (P = 0.76). The magnitudes of these CVs are below those obtained under very controlled circumstances (30).

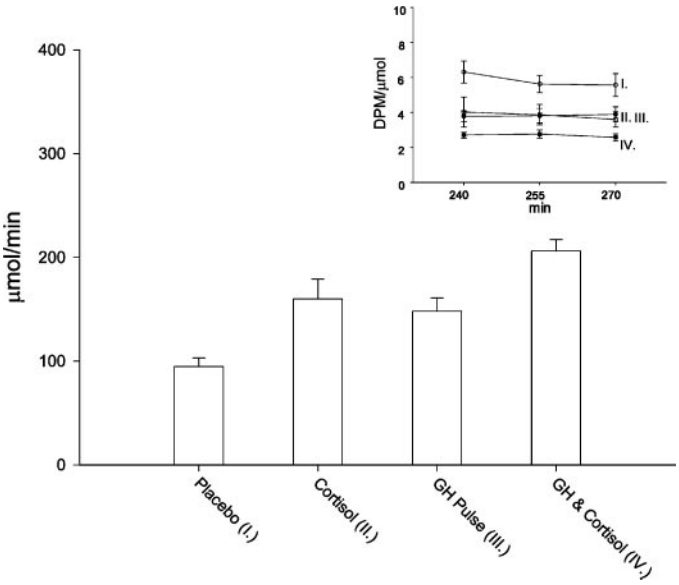

Fig. 4. Systemic palmitate fluxes at t = 240–270 min. IV > III, and II > I, P < 0.05. *Inset*: palmitate specific activities during the 4 treatment arms in the interval 240–270 min (see text for statistics).

Glucose, glucose infusion rate, and blood flow. Plasma glucose tended to increase upon institution of the pancreatic clamp and tended to be higher in III (120 ± 11 mg/dl) and IV (110 ± 9 mg/dl) than in I (93 ± 11 mg/dl) and II (94 ± 6 mg/dl); however, no statistical difference was observed. The amount of glucose needed to maintain plasma glucose above 80 mg/dl was very modest and identical in either of the study arms (P = 0.75).

SMBF did not change over time during the experiments, and no difference was observed between treatments. The average blood flow was 3.2 ± 0.5 ml·100 ml tissue^{-1}·min^{-1}.

Additive effects of GH and cortisol on lipolysis. Any additive effects (E) of GH and cortisol on lipolysis can be evaluated by comparing the theoretically deduced additive product with the observed

$$(E_{cortisol} + E_{GH}) - E_{placebo} = E_{cortisol\&GH}$$

When these theoretically deduced additive parameters for FFA R_a, systemic FFA, serum glycerol, ISMG, and femoral/abdominal interstitial glycerol were compared with the observed findings during IV (Fig. 5), we found no difference, indicating that cortisol potentiates GH-induced lipolysis.

DISCUSSION

The aim of the present study was to assess acute lipolytic actions of GH and cortisol at the whole body level and in subcutaneous adipose tissue and skeletal muscle, since these hormones are cosecreted during fasting (3, 33), exercise (11, 12, 26, 29), and other kinds of perceived stress (43).

In line with our previous studies involving GH and cortisol, we observed an increase in the circulating lipolytic parameters FFA and glycerol upon stimulation. Interstitial adipose tissue lipolysis, although technical difficulties prevailed, also showed similar results to those found previously. The novelty of this study clearly lies in the additive effects of the two hormones.

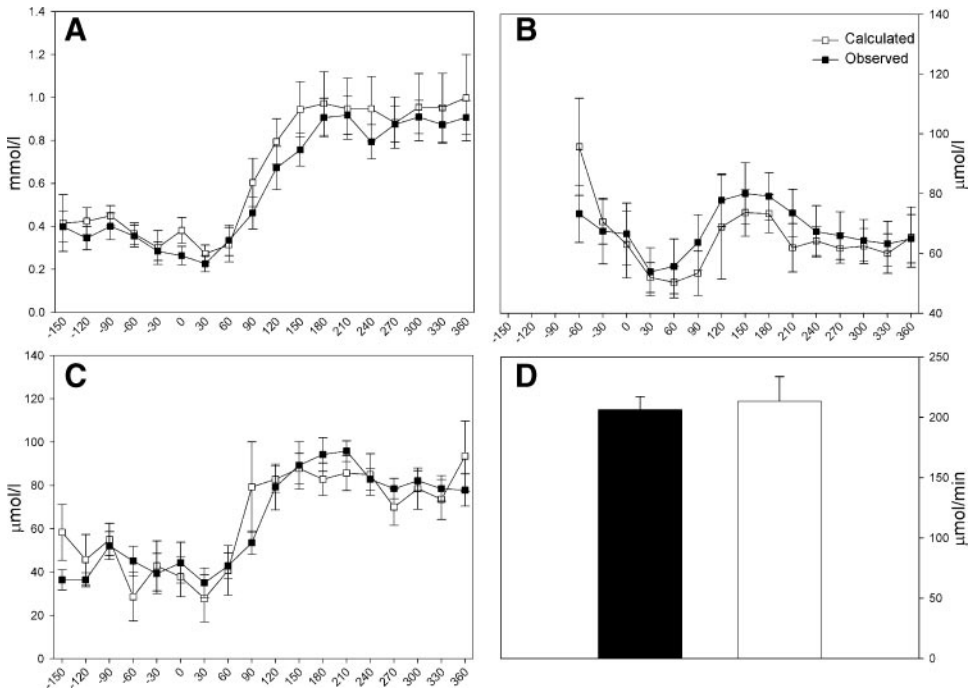

Fig. 5. Observed (exposure IV) and calculated additive effects of hydrocortisone + GH administration. *A*: serum FFA. *B*: serum glycerol. *C*: interstitial muscle glycerol. *D*: systemic palmitate fluxes.

As early as the 1960s, Fain and coworkers (14, 15) showed additive lipolytic effects of GH coincubated with DEX after 2 h. More recently, this has been described by Yip and Goodman (44), further emphasizing the possible additive mode of action.

As of now, the exact mechanisms of the lipolytic properties of GH and cortisol are not known. It has been the general perception that GH enhances the effects of catecholamines, and the aforementioned in vitro studies have to some extent confirmed this.

Recently, we (20) have shown that GH-mediated lipolysis is maximally induced at a dose of 3 μg/kg. This dose is comparable to the dose chosen in the present study. Our finding that markers of lipolysis appear to be increased in an additive manner during combined stimulation with GH and cortisol suggests that the mechanisms of action for these two hormones are via two distinct pathways. This corresponds favorably with the findings of Yip and Goodman (44), wherein GH and DEX reduced the inhibitory G-protein α_2-receptors, thereby increasing sensitivity to adrenergic β-receptor-mediated lipolysis. Whether the isolated stimulations of GH or cortisol have any effect on the α_2-receptors was not addressed. In the same study, selective blockade of cAMP production reduced the lipolytic potential of GH plus DEX, indicating that the lipolytic pathways involved are mediated by increased cAMP production. The authors, however, found that lipolysis induced by GH or DEX given alone was unaffected by inhibition of cAMP production. These results are difficult to reconcile but may indicate either that GH and DEX (cortisol) work via more than one pathway or that only combined GH plus DEX increases cAMP production, whereas when given independently they work via different pathways.

Intramuscular TAG breakdown has been suggested to be an important source of fuel in skeletal muscle. Increased interstitial glycerol has been interpreted by many authors as a marker of intramuscular lipolysis (13, 18, 39). However, the presence of glycerol kinase in skeletal muscle, enabling the use of glycerol derived from TAG to undergo reacetylation, rather than glucose, may distort results. Until recently, there has been no indication of its presence. Coppack et al. (8) and Jensen et al. (23) have, however, challenged this conception. In addition, glycerol observed in interstitial skeletal muscle could be interpreted as glycerol derived from the general circulation, although this seems unlikely in our study because the ratio of serum glycerol to interstitial muscle glycerol was not constant even though no change in skeletal muscle blood flow was observed. Furthermore, the concentrations observed in interstitial muscle glycerol were ~50% higher during baseline than those in serum glycerol, a finding hardly explained by assay differences.

We did not observe any changes in glucose infusion in either of the arms, indicating that no, or very minute, insulin resistance was instituted with the short duration of these stimulations, in line with previous observations (38). This corresponds to previous findings with GH (32) and cortisol (10), whereas prolonged exposure to both hormones is known to generate insulin resistance. Clearly, the exact pathways through which these two key metabolic hormones influence lipid metabolism during normal physiological excursions deserves further exploration. The finding that GH causes translocation of perilipin in rat adipocytes (7), and therefore renders the lipid droplet more accessible to HSL, could very well be an important regulatory mechanism.

ACKNOWLEDGMENTS

We thank Annette Mengel for excellent technical assistance. Roche Diagnostics kindly donated the microdialysis catheters.

GRANTS

This study was supported by an unrestricted grant from Novo Nordisk as well as a grant from the University of Aarhus Research Foundation, the Danish Center for Growth and Regeneration, and the Danish Diabetes Association.

REFERENCES

1. **Abumrad NN, Rabin D, Diamond MP, and Lacy WW.** Use of a heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. *Metabolism* 30: 936–940, 1981.
2. **Arner P and Bolinder J.** Microdialysis of adipose tissue. *J Intern Med* 230: 381–386, 1991.
3. **Bergendahl M, Vance ML, Iranmanesh A, Thorner MO, and Veldhuis JD.** Fasting as a metabolic stress paradigm selectively amplifies cortisol secretory burst mass and delays the time of maximal nyctohemeral cortisol concentrations in healthy men. *J Clin Endocrinol Metab* 81: 692–699, 1996.
4. **Brasaemle DL, Levin DM, Adler-Wailes DC, and Londos C.** The lipolytic stimulation of 3T3-L1 adipocytes promotes the translocation of hormone-sensitive lipase to the surfaces of lipid storage droplets. *Biochim Biophys Acta* 1483: 251–262, 2000.
5. **Cahill GF.** Starvation in man. *Clin Endocrinol Metab* 5: 397–415, 1976.
6. **Carstensen E and Yudkin JS.** Platelet catecholamine concentrations after short-term stress in normal subjects. *Clin Sci (Lond)* 86: 35–41, 1994.
7. **Clifford GM, Kraemer FB, Yeaman SJ, and Vernon RG.** Translocation of hormone-sensitive lipase and perilipin upon lipolytic stimulation during the lactation cycle of the rat. *Metabolism* 50: 1264–1269, 2001.
8. **Coppack SW, Persson M, Judd RL, and Miles JM.** Glycerol and nonesterified fatty acid metabolism in human muscle and adipose tissue in vivo. *Am J Physiol Endocrinol Metab* 276: E233–E240, 1999.
9. **Divertie GD, Jensen MD, and Miles JM.** Stimulation of lipolysis in humans by physiological hypercortisolemia. *Diabetes* 40: 1228–1232, 1991.
10. **Djurhuus CB, Gravholt CH, Nielsen S, Mengel A, Christiansen JS, Schmitz OE, and Møller N.** Effects of cortisol on lipolysis and regional interstitial glycerol levels in humans. *Am J Physiol Endocrinol Metab* 283: E172–E177, 2002.
11. **Duclos M, Gouarne C, and Bonnemaison D.** Acute and chronic effects of exercise on tissue sensitivity to glucocorticoids. *J Appl Physiol* 94: 869–875, 2003.
12. **Ehrnborg C, Lange KH, Dall R, Christiansen JS, Lundberg PA, Baxter RC, Boroujerdi MA, Bengtsson BA, Healey ML, Pentecost C, Longobardi S, Napoli R, and Rosen T.** The growth hormone/insulin-like growth factor-I axis hormones and bone markers in elite athletes in response to a maximum exercise test. *J Clin Endocrinol Metab* 88: 394–401, 2003.
13. **Enoksson S, Degerman E, Hagstrom-Toft E, Large V, and Arner P.** Various phosphodiesterase subtypes mediate the in vivo antilipolytic effect of insulin on adipose tissue and skeletal muscle in man. *Diabetologia* 41: 560–568, 1998.
14. **Fain JN.** Inhibition of lipolytic action of growth hormone and glucocorticoid by ultraviolet and x-radiation. *Science* 157: 1062–1064, 1967.
15. **Fain JN, Kovacev VP, and Scow RO.** Effect of growth hormone and dexamethasone on lipolysis and metabolism in isolated fat cells of the rat. *J Biol Chem* 240: 3522–3529, 1965.
16. **Frayn KN, Coppack SW, Fielding BA, and Humphreys SM.** Coordinated regulation of hormone-sensitive lipase and lipoprotein lipase in human adipose tissue in vivo: implications for the control of fat storage and fat mobilization. *Adv Enzyme Regul* 35: 163–178, 1995.
17. **Gravholt CH, Schmitz O, Simonsen L, Bülow J, Christiansen JS, and Møller N.** Effects of a physiological GH pulse on interstitial glycerol in abdominal and femoral adipose tissue. *Am J Physiol Endocrinol Metab* 277: E848–E854, 1999.
18. **Hagstrom-Toft E.** Microdialysis for the assessment of catecholamine-induced lipolysis in adipose and skeletal muscle tissue. *Adv Pharmacol* 42: 634–638, 1998.
19. **Hagstrom-Toft E, Enoksson S, Moberg E, Bolinder J, and Arner P.** Absolute concentrations of glycerol and lactate in human skeletal muscle, adipose tissue, and blood. *Am J Physiol Endocrinol Metab* 273: E584–E592, 1997.
20. **Hansen TK, Gravholt CH, Ørskov H, Rasmussen MH, Christiansen JS, and Jorgensen JO.** Dose dependency of the pharmacokinetics and acute lipolytic actions of growth hormone. *J Clin Endocrinol Metab* 87: 4691–4698, 2002.
21. **Holm C, Osterlund T, Laurell H, and Contreras JA.** Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. *Annu Rev Nutr* 20: 365–393, 2000.
22. **Jansson PA, Smith U, and Lönnroth P.** Interstitial glycerol concentration measured by microdialysis in two subcutaneous regions in humans. *Am J Physiol Endocrinol Metab* 258: E918–E922, 1990.
23. **Jensen MD, Chandramouli V, Schumann WC, Ekberg K, Previs SF, Gupta S, and Landau BR.** Sources of blood glycerol during fasting. *Am J Physiol Endocrinol Metab* 281: E998–E1004, 2001.
24. **Jensen MD, Rogers PJ, Ellman MG, and Miles JM.** Choice of infusion-sampling mode for tracer studies of free fatty acid metabolism. *Am J Physiol Endocrinol Metab* 254: E562–E565, 1988.
25. **Jeukendrup AE.** Regulation of fat metabolism in skeletal muscle. *Ann NY Acad Sci* 967: 217–235, 2002.
26. **Kjaer M, Bangsbo J, Lortie G, and Galbo H.** Hormonal response to exercise in humans: influence of hypoxia and physical training. *Am J Physiol Regul Integr Comp Physiol* 254: R197–R203, 1988.
27. **Lloyd B, Burrin J, Smythe P, and Alberti KG.** Enzymic fluorometric continuous-flow assays for blood glucose, lactate, pyruvate, alanine, glycerol, and 3-hydroxybutyrate. *Clin Chem* 24: 1724–1729, 1978.
28. **Lönnroth P and Strindberg L.** Validation of the "internal reference technique" for calibrating microdialysis catheters in situ. *Acta Physiol Scand* 153: 375–380, 1995.
29. **Luger A, Deuster PA, Kyle SB, Gallucci WT, Montgomery LC, Gold PW, Loriaux DL, and Chrousos GP.** Acute hypothalamic-pituitary-adrenal responses to the stress of treadmill exercise. Physiologic adaptations to physical training. *N Engl J Med* 316: 1309–1315, 1987.
30. **Maheux P, Azhar S, Kern PA, Chen YD, and Reuven GM.** Relationship between insulin-mediated glucose disposal and regulation of plasma and adipose tissue lipoprotein lipase. *Diabetologia* 40: 850–858, 1997.
31. **Miles JM, Ellman MG, McClean KL, and Jensen MD.** Validation of a new method for determination of free fatty acid turnover. *Am J Physiol Endocrinol Metab* 252: E431–E438, 1987.
32. **Møller N, Jorgensen JO, Schmitz O, Møller J, Christiansen J, Alberti KG, and Ørskov H.** Effects of a growth hormone pulse on total and forearm substrate fluxes in humans. *Am J Physiol Endocrinol Metab* 258: E86–E91, 1990.
33. **Møller N and Norrelund H.** The role of growth hormone in the regulation of protein metabolism with particular reference to conditions of fasting. *Horm Res* 59, *Suppl* 1: 62–68, 2003.
34. **Møller N, Schmitz O, Pørksen N, Møller J, and Jorgensen JO.** Dose-response studies on the metabolic effects of a growth hormone pulse in humans. *Metabolism* 41: 172–175, 1992.
35. **Ørskov H, Thomsen HG, and Yde H.** Wick chromatography for rapid and reliable immunoassay of insulin, glucagon and growth hormone. *Nature* 219: 193–195, 1968.
36. **Ottosson M, Lönnroth P, Björntorp P, and Eden S.** Effects of cortisol and growth hormone on lipolysis in human adipose tissue. *J Clin Endocrinol Metab* 85: 799–803, 2000.
37. **Owen OE, Smalley KJ, D'Alessio DA, Mozzoli MA, and Dawson EK.** Protein, fat, and carbohydrate requirements during starvation: anaplerosis and cataplerosis. *Am J Clin Nutr* 68: 12–34, 1998.
38. **Plat L, Byrne MM, Sturis J, Polonsky KS, Mockel J, Féry F, and Van Cauter E.** Effects of morning cortisol elevation on insulin secretion and glucose regulation in humans. *Am J Physiol Endocrinol Metab* 270: E36–E42, 1996.
39. **Rosdahl H, Hamrin K, Ungerstedt U, and Henriksson J.** Metabolite levels in human skeletal muscle and adipose tissue studied with microdialysis at low perfusion flow. *Am J Physiol Endocrinol Metab* 274: E936–E945, 1998.
40. **Scheig R.** Diseases of lipid metabolism. In: *Diseases of Metabolism—Genetics and Metabolism*, edited by Bondy PK and Rosenberg LE. Philadelphia, PA: Saunders, 1974, p. 341–415.
41. **Scheller D and Kolb J.** The internal reference technique in microdialysis: a practical approach to monitoring dialysis efficiency and to calculating tissue concentration from dialysate samples. *J Neurosci Methods* 40: 31–38, 1991.
42. **Spriet LL.** Regulation of skeletal muscle fat oxidation during exercise in humans. *Med Sci Sports Exerc* 34: 1477–1484, 2002.
43. **Stubbs PJ, Laycock J, Alaghband-Zadeh J, Carter G, and Noble MI.** Circulating stress hormone and insulin concentrations in acute coronary syndromes: identification of insulin resistance on admission. *Clin Sci (Colch)* 96: 589–595, 1999.
44. **Yip RG and Goodman HM.** Growth hormone and dexamethasone stimulate lipolysis and activate adenylyl cyclase in rat adipocytes by selectively shifting Gi alpha2 to lower density membrane fractions. *Endocrinology* 140: 1219–1227, 1999.