Catecholamine and insulin control of lipolysis in subcutaneous adipose tissue during long-term diet-induced weight loss in obese women

Katrien Koppo,1,2 Michaela Siklová-Vitková,1,3 Eva Klimčáková,1,3 Jan Polák,1,3 Marie A. Marques,3,4 Michel Berlan,3,4 Johan Van de Voorde,2 Jens Bulow,5 Dominique Langin,3,4,6 Isabelle de Glisezinski,3,4,7 and Vladimir Stich1,3

1Department of Sports Medicine, Third Faculty of Medicine, Charles University, Prague, Czech Republic; 2Department of Pharmacology, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium; 3Franco-Czech Laboratory of Clinical Research on Obesity, Institut National de la Santé et de la Recherche Médicale (INSERM) and Third Faculty of Medicine of the Charles University, Prague, Czech Republic; 4Laboratoire de Recherches sur les Obésités, Institut des Maladies Métaboliques et Cardiovasculaires (12MC), UMR 1048 INSERM, Université Paul Sabatier, Toulouse, France; 5Department of Clinical Physiology, Bispebjerg Hospital and Institute of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark; and Departments of 6Clinical Biochemistry and 7Sports Medicine, Centre Hospitalier Universitaire, Toulouse, France

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Dietary calorie restriction is an essential part of the weight-reducing treatment of obese patients. Besides body weight reduction, hypocaloric diet induces an improvement of insulin resistance with respect to carbohydrate metabolism of obese patients (25, 26). Because several studies suggest that whole body insulin resistance is related to an impairment in the regulation of lipolysis (3, 7, 12, 19), it is to be expected that a hypocaloric diet may be associated with a beneficial modification of the impaired lipolytic responsiveness.

Subcutaneous adipose tissue (SCAT) lipolysis is regulated by a complex interplay of inhibitory and stimulatory pathways. Catecholamines are important regulators of lipolysis. They bind to G protein-coupled adrenergic receptors (ARs) controlling intracellular cyclic AMP levels and thus lipolysis. Stimulation of lipolytic β-ARs coupled to the stimulatory Gs proteins increases cAMP production, whereas stimulation of the antilipolytic α2-ARs that are coupled to Gi proteins decreases cAMP production. It has been shown that the responsiveness of lipolysis to β-adrenergic stimulation is impaired in obese individuals (3, 12) and that obese subjects show a greater α2-AR activity, leading to an impaired exercise-induced lipolysis when compared with lean subjects (22). Furthermore, a low-calorie diet (LCD) reduced the α2-AR mediated antilipolytic action in obese subjects (23). However, the results regarding the effect of a hypocaloric diet on the responsiveness to the β-adrenergic stimulation of lipolysis are less consistent; some studies report an improvement after a very low-calorie diet (VLCD) (2, 21), whereas others find no changes in lipolytic responsiveness (11, 23). This discrepancy may be related to differences in duration of the diet and/or magnitude of the diet-induced calorie restriction. How lipolysis and, more specifically, the functional balance between the adrenergic β- and α2-dependent pathways change during a long-term dietary intervention has not been investigated yet. Therefore, the first aim of this study was to investigate the adrenergic regulation of lipolysis during different phases of a multiple-phase dietary intervention.
of the diet and a decrease under the prediet level after an extended period of weight loss (15). Therefore, in light of these results, it is not inconceivable that lipolysis during a multiple-stage dietary intervention behaves dynamically, with the underlying mechanisms remaining to be clarified.

Besides catecholamines, insulin is another important regulator of lipolysis. It acts mainly through activation of phosphodiesterase 3B that reduces cAMP levels, which controls the activity of cAMP-dependent protein kinase and in turn controls the activation state of lipases and lipid droplet-associated proteins (17). It was shown that subjects who were less insulin sensitive, e.g., healthy relatives of type 2 diabetes patients, were also less sensitive to the antilipolytic effect of insulin in SCAT (7). Furthermore, it was reported that, in upper body obese insulin-resistant subjects, the adrenergic stimulation of lipolysis is reduced (20). Therefore, the second aim of the study was to examine whether during a multiple-stage diet the improvement in whole body insulin sensitivity with respect to carbohydrate metabolism coincided with an increased antilipolytic effect of insulin. A hyperinsulinemic euglycemic clamp (HEC) was performed to assess the association between diet-induced changes of whole body insulin sensitivity and the antilipolytic effect of insulin on the adrenergic regulation of lipolysis.

To investigate the diet-induced impact on the antilipolytic effect of insulin, the adrenergic responsiveness, and the interplay between the two pathways, SCAT lipolysis was investigated by means of the microdialysis technique in a group of obese women who were enrolled in a 6-mo multiple-phase dietary intervention. At four time points during the intervention [prediet, VLCD, LCD, and weight maintenance (WM) phase], a HEC was performed for 3 h. Glycerol release was studied in a probe perfused with Ringer solution. Before and during the last 30 min of the clamp, adrenergic compounds were infused directly into the microdialysis probes. In one probe, the ARs of the surrounding fat cells were stimulated by infusion of adrenaline, whereas in another probe besides adrenaline, phentolamine was infused to block the α-ARs of the surrounding fat cells.

MATERIALS AND METHODS

Subjects. Eight obese premenopausal women, highly motivated for a weight reduction program, were recruited from an outpatient obesity clinic (Table 1). They all had stable weights during the 3 mo that preceded the study. Exclusion criteria were hypertension, diabetes, hyperlipidemia treated by drugs, drug-treated obesity, drug or alcohol abuse, pregnancy, or participation in other studies. All of the subjects were fully informed about the aims and the protocol of the study and signed an informed consent form approved by the ethics committee of the Third Faculty of Medicine of the Charles University of Prague, Czech Republic.

Dietary intervention. During the first month of the dietary intervention program, the subjects received an 800 kcal/day VLCD (liquid formula diet; Redita, Promil, Czech Republic). During the next 2 mo, a LCD was designed to provide 600 kcal/day less than the individually estimated energy requirement based on an initial resting metabolic rate multiplied by 1.3 (the coefficient of correction for physical activity level). The final 3 mo consisted of a WM diet, during which the subjects kept a stable weight. The subjects consulted a dietician once/wk during the first 3 mo and once/mo during the WM phase. They provided a written 3-day dietary record at each consultation.

Experimental protocol. On four occasions [i.e., before the start of the dietary intervention (baseline) and at the end of the VLCD, LCD, and WM phases], the subjects entered the laboratory at 8 AM, after an overnight fast. A complete clinical investigation was performed, anthropometric parameters were measured, and body composition was determined with multifrequency bioimpedance (Bodystat QuadScan 4000; Bodystat, Isle of Man, British Isles). Subsequently, the subjects were placed in a semirecumbent position. Two indwelling polyethylene catheters were inserted, one into an antecubital vein (for insulin and glucose infusion) and one into a dorsal vein of the ipsilateral hand (for blood sampling). The hand was kept warm to provide arterIALIZATION of venous blood. Then, three microdialysis probes (Carnegie Medicine, Stockholm, Sweden) of 20 × 0.5 mm and 20,000-MW cutoff were inserted percutaneously after epidermal anesthesia (200 μl of 1% lidocaine; Roger-Bellon, Neuiilly-sur-Seine, France) into the SCAT at a distance of 10 cm from the umbilicus. The probes were connected to a microperefusion pump (Harvard Apparatus; S.A.R.L., Les Ulis, France) and perfused at a rate of 2.5 μl/min with Ringer solution. After the 60-min equilibration period, all the probes were perfused for 30 min with Ringer solution. During the following 30 min, a first lipolytic challenge was performed (perfusion 1): probe 1 was perfused with Ringer solution (served mainly to assess the response to hyperinsulinemia), probe 2 was perfused with 10 μmol/l adrenaline (a non-selective adrenergic receptor agonist), and probe 3 was perfused with adrenaline plus 100 μmol/l phentolamine (an α1,-adrenergic receptor antagonist). After this 30-min lipolytic challenge, the HEC was started, and all probes were perfused with Ringer solution again. At 150 min after initiation of HEC, a second lipolytic challenge identical to that before the HEC was performed.
(perfusion 2), i.e., a 30-min period of perfusion with adrenaline (probe 2) or adrenaline plus phentolamine (probe 3). As shown previously by our group (24), this time lapse between the adrenaline infusions is necessary to avoid desensitization of catecholamine-induced lipolysis. Throughout the protocol, fractions of the outgoing dialysate from the three probes were collected every 10 min to measure glycerol concentration. The urea concentration in the outgoing dialysate was determined to estimate whether major changes in the subcutaneous adipose tissue blood flow (ATBF) occurred during the experiment (13). Blood was drawn before the clamp and every hour during the HEC to determine plasma concentrations of glucose, glycerol, nonesterified fatty acids, (NEFA) and free insulin. A schematic representation of the protocol is shown in Fig. 1.

**HEC.** HEC was performed according to the DeFronzo method (5). Priming plus continuous infusion of crystalline human insulin (Actrapid Hum; Novo, Bagsvaerd, Denmark), 40 mU·m² body area⁻¹·min⁻¹, was given for 180 min. Euglycemia (the fasting blood glucose concentration) was maintained by a variable 20% glucose infusion. The infusion rate was determined by measuring arterialized plasma glucose every 5 min (Beckman glucose Analyzer; Beckman Coulter, Fullerton, CA).

**Drugs and biochemical determinations.** Adrenaline hydrochloride was obtained from Stallergenes (Antony, France) and phentolamine methanesulfonate (Regitine) from Novartis Pharma (Rueil-Malmaison, France). Glycerol in dialysate and in plasma was analyzed by an enzymatic method (Sigma, St. Louis, MO). Plasma glucose and NEFA levels were determined with a glucose hexokinase technique (Konelab 60i; Labsystems, Konelab, Finland) and an enzymatic procedure (Wako; Unipath, Dardilly, France), respectively. Plasma insulin concentrations were measured using a chemiluminescent immunoassay (Immulite 2000 Insulin; DPC Czech sro, Brno, Czech Republic). Urea determination in the dialysate was measured using an enzymatic method (Urea Kit S1000; Biomerieux, Marcy-L’Etoile, France). Plasma urea concentrations were determined using routine methods.

**Statistical analysis.** All values are presented as means ± SE. Statistical evaluation of the data was performed using ANOVA for repeated measurements with at least significant difference post hoc test (SPSS statistical software, version 19; SPSS, Chicago, IL). The comparison of the dynamic curves representing the responses during HEC at different time points of the intervention (i.e., the responses of the plasma variables and the dialysate glycerol levels evaluated in the Ringer probe during HEC) was analyzed using a two-way ANOVA, with “time point of the intervention” and “time during the HEC” as main factors. To quantify the magnitude of the adrenaline-induced SCAT lipolysis, the area under the curve (AUC; i.e., the integral of the total increase above the baseline value) of the dialysate glycerol was calculated. Significance was determined at P < 0.05.

**RESULTS**

**Anthropometric and plasma parameters of the subjects during the different phases of the diet.** Prediet values and values measured at different phases during the weight reduction program (i.e., at the end of a 1-mo 800 kcal/day VLCD, at the end of a 2-mo LCD with 600 kcal less than the calculated daily energy requirements, and at the end of a 3-mo WM diet) are reported in Table 1. The subjects’ body weight progressively decreased during VLCD and LCD and stabilized during WM. The loss in weight was due mainly to a decrease in fat mass.

Compared with the prediet baseline values, plasma insulin was significantly lower at the end of VLCD, LCD, and WM (Table 1). During HEC, insulin infusion induced a steady-state insulin level (88.5 ± 3.4 mU/I before the diet and 67.3 ± 3.7, 80.5 ± 9.0, and 70.3 ± 4.8 mU/I at the end of the VLCD, LCD, and WM diet, respectively). The subjects were clamped at their individual fasting glucose levels (i.e., 5.5 ± 0.1 mmol/I before the diet and 5.3 ± 0.3, 5.6 ± 0.2, and 6.0 ± 0.3 mmol/I at the end of the VLCD, LCD, and WM diet, respectively). Insulin sensitivity of the subjects was assessed by the glucose disposal rate and by the quantitative insulin sensitivity check index (QUICKI) (14). The QUICKI values were increased significantly at the end of VLCD and remained elevated at the end of the LCD and WM diet. A similar pattern was observed for the glucose disposal rate, but at the end of VLCD the increase was not significant, although it was significantly elevated at the end of the LCD and WM diet (Table 1).

Compared with the prediet baseline value, no change in plasma NEFA was observed at the end of VLCD. However, baseline plasma NEFA decreased significantly at the end of the LCD and WM diet. Before and at all subsequent phases of the diet, plasma NEFA decreased during HEC. The magnitude of decrease in plasma NEFA during HEC was similar in the different phases of the diet (Fig. 2A). Regarding plasma glycerol, lower values were observed at the end of VLCD compared with the prediet baseline values, but the decrease was not significant. At the end of LCD and WM diet, significantly lower plasma glycerol values were observed compared with the prediet baseline values. Similar to plasma NEFA, the dietary intervention did not affect the magnitude of decrease in plasma glycerol during HEC at any phase of the diet (Fig. 2B).

**Dialysate glycerol concentration during the different phases of the diet.** The dialysate glycerol concentration was evaluated in the control Ringer probe. The baseline levels of dialysate glycerol (measured before the start of HEC) were significantly lower at all phases of the diet compared with the prediet levels (i.e., 77.3 ± 11.8 µmol/I before the diet vs. 36.8 ± 4.3, 41.0 ± 7.7, and 34.4 ± 2.3 µmol/I at the end of VLCD, LCD, and WM diet, respectively). Before and at all subsequent phases of the diet, dialysate glycerol decreased progressively during HEC (i.e., by 30 ± 11% before the diet, 37 ± 4% at the end of the VLCD, 35 ± 12% at the end of the LCD, and 30 ± 17% at the end of the WM diet), and this decrease was not significantly different between the different phases of the diet (Fig. 3).
Lipolytic response to adrenaline (with or without phentolamine) perfusion in SCAT during the different phases of the diet. During the perfusion of adrenaline performed before HEC, the increase in dialysate glycerol concentration, expressed as AUC, was significantly higher at the end of VLCD and LCD than before the start of the diet (Fig. 4A). At the end of WM, AUCs returned to the levels similar to those observed before the diet. During the last 30 min of HEC, a second perfusion was performed. The increases in dialysate glycerol concentration were lower than those observed during the first perfusion, the difference being significant at the end of VLCD, LCD, and WM.

During the perfusion of adrenaline plus phentolamine performed before HEC, a pattern similar (i.e., a higher increase in dialysate glycerol at the end of VLCD and LCD) to that during the adrenaline perfusion was observed, but the differences did not reach significance (Fig. 4B). For the second perfusion during HEC, AUCs were significantly lower than that observed during the first perfusion before and at all subsequent phases of the diet.

During the perfusion of adrenaline plus phentolamine performed before HEC, a pattern similar (i.e., a higher increase in dialysate glycerol at the end of VLCD and LCD) to that during the adrenaline perfusion was observed, but the differences did not reach significance (Fig. 4B). For the second perfusion during HEC, AUCs were significantly lower than that observed during the first perfusion before and at all subsequent phases of the diet.
To estimate the importance of the $\alpha_2$-adrenergic pathway in the adrenaline-induced lipolysis, the changes in dialysate glycerol initiated by adrenaline infusion alone and in combination with phentolamine were compared before HEC. Before the start of the diet, significantly higher AUCs were observed when adrenaline was combined with phentolamine (Fig. 4C). At the end of the VLCD and LCD, this difference disappeared, and similar AUCs were observed between the two probes. At the end of WM, significantly higher AUCs reappeared in the probe perfused with adrenaline plus phentolamine.

Modifications of regional ATBF. Plasma levels of urea did not change throughout the intervention (Table 1). The baseline values of urea in the dialysate (measured during 30 min before the start of HEC) did not significantly differ between the four phases of the diet (0.75 ± 0.20 mmol/l before the diet, 0.57 ± 0.14 mmol/l at the end of the VLCD, 0.75 ± 0.15 mmol/l at the end of the LCD, and 0.68 ± 0.15 at the end of WM). Similarly, no significant differences were observed between the respective phases of the diet when the urea concentrations in the dialysate measured during the local perfusion with adrenaline (0.58 ± 0.06 mmol/l before the diet, 0.31 ± 0.07 mmol/l at the end of the VLCD, 0.70 ± 0.11 mmol/l at the end of the LCD, and 0.60 ± 0.14 at the end of WM) or adrenaline plus phentolamine (0.59 ± 0.09 mmol/l before the diet, 0.53 ± 0.16 mmol/l at the end of the VLCD, 0.65 ± 0.13 mmol/l at the end of the LCD, and 0.65 ± 0.14 at the end of WM) were considered. The results show that the experimental setup did not induce substantial changes in ATBF.

DISCUSSION

The major finding of this study is that during a 6-mo dietary intervention, leading to an improvement in insulin sensitivity with respect to carbohydrate metabolism, the lipolytic responsiveness to adrenergic stimulation evaluated by in situ microdialysis in SCAT was enhanced during the first part of the dietary intervention, representing the dynamic phase of weight loss, with a decrease in the $\alpha_2$-AR antilipolytic effect, and returned back to the prediet levels during the subsequent WM period. The antilipolytic effect of insulin was similar in the various phases of the dietary intervention, because the time courses of the decrease in dialysate glycerol response during HEC did not significantly differ between the respective phases of the intervention.

The lipolytic response to adrenaline stimulation mediated by simultaneous stimulation of lipolytic $\beta$-ARs and antilipolytic $\alpha_2$-ARs was enhanced during the negative energy balance period represented by the VLCD and the subsequent LCD. Then, adrenergic responsiveness returned to the prediet values during the WM phase, which represents a new steady state of energy balance. Comparison of the adrenaline-induced lipolytic responses measured in the probe with adrenaline alone and in the probe with adrenaline and phentolamine, i.e., with a blockade of $\alpha_2$-ARs, revealed that the pronounced $\alpha_2$-mediated antilipolytic effect observed in the prediet condition was blunted during the phases of negative energy balance (VLCD and LCD) and reappeared during the WM phase. Taking these longitudinal results into consideration, it may be concluded that during the dynamic phase of negative energy balance the responsiveness to the $\beta$-adrenergic stimulation of lipolysis is maintained and that to the $\alpha_2$-mediated antilipolytic effect is reduced. As a result, the lipolytic response to adrenaline increases during the VLCD and LCD and comes back to the prediet values at the WM phase.

These results are in agreement with previous studies that were limited to the exploration of the adrenergic regulation of lipolysis during a single phase of the LCD. In fact, Barbe et al. (2) reported an increase in the in situ lipolytic response to $\beta$-adrenergic stimulation during 28 days of VLCD. Similarly, isoprenaline-induced lipolysis in isolated adipocytes was also increased during VLCD (21). Concerning the $\alpha_2$-AR-mediated regulation, the results of the present study are in agreement with the results of Stich et al. (23), which showed that 3 mo of LCD induced a decrease of the in situ antilipolytic $\alpha_2$-mediated action of catecholamines.

Hypocaloric diets induce a marked improvement in the insulin resistance of obese individuals, and this improvement seems to take place in the initial weeks of the diet before the subjects lose a substantial amount of weight (1). Furthermore, it was shown that whole body insulin resistance and/or upper body obesity are related to impairments in the regulation of lipolysis either in vivo or in vitro. In fact, an impaired responsiveness of lipolysis to $\beta$-adrenergic stimulation was reported in isolated subcutaneous adipocytes of obese men with insulin resistance (19) and in vivo in whole body and/or SCAT of obese men (3) and women (12). Furthermore, it was shown that the obese subjects have a higher $\alpha_2$-AR-mediated antilipolytic responsiveness, leading to an impaired exercise-induced lipid mobilization when compared with lean subjects (22). Therefore, we expected that the current dietary intervention, which led to an improvement of whole body insulin sensitivity, would result in a beneficial modification of the impaired lipolytic responsiveness. After VLCD and LCD, the adrenaline-induced SCAT lipolysis increased significantly, and the $\alpha_2$-AR-mediated antilipolytic action decreased. However, the enhancing effect of calorie restriction disappeared with WM, and the SCAT lipolysis returned back to the original prediet levels despite maintenance of improved insulin sensitivity. Therefore, it may be hypothesized that the changes in lipolytic responsiveness are sensitive to the energy restriction rather than to the total weight loss and/or insulin sensitivity. During WM, where a new stage of energy balance was achieved at a lower weight, the diet-induced changes in adrenergic regulation disappeared. Similar findings were reported in previous studies that examined the evolution of adipose tissue expression of genes related to metabolism or to immune function (e.g., see Refs. 1, 4, and 25). These studies demonstrated that the diet-induced changes in metabolic and immune gene expression profiles were strongly dependent on the phases of the diet; i.e., they were altered during a phase of energy deficiency and restored when the energy restriction was terminated, even when the weight lost was not regained. Furthermore, in a recent study in mice, it was shown that the adipose tissue lipolysis also depended on the phase of the diet (15). The observed pattern was similar to that of our study, i.e., an increase in the lipolytic response during the initial phase of the diet and a gradual decrease toward the prediet levels during the subsequent phase of the diet.

As mentioned above, our dietary intervention led to an improvement in whole body insulin sensitivity with respect to carbohydrate metabolism. Because insulin is known as a powerful antilipolytic agent, it could be hypothesized that an
improvement in whole body insulin sensitivity would be paral-leled by an improvement in the sensitivity to the insulin-mediated antilipolytic action. However, no significant differences in the magnitude of the decrease of dialysate glycerol during HEC were observed when the different phases of the diet were compared. This could be due to the high dose of insulin that was used during the HEC in the present study. However, in the study of Hagström-Toft et al. (10), where SCAT lipolysis was studied in situ during a two-step HEC (with lower insulin levels during the 1st step) in obese women before and after VLCD, similar results were reported; i.e., the glycerol release in SCAT decreased during HEC, and the magnitude of the decrease was similar before and after the VLCD. According to the above-mentioned findings, the sensitivity to the antilipolytic action of insulin in adipose tissue during the dietary intervention seems to not be directly related to the evolution of the whole body insulin sensitivity with respect to carbohydrate metabolism. It was shown that hyperinsulinemia reduces the lipolytic effect of catecholamines both in vitro (6) and in vivo (24). These authors suggested insulin-dependent activation of phosphodiesterase, phosphorylation of the G protein linked to the β-AR, and a modification of the β/α2-AR balance in favor of the α2-AR antilipolytic pathway as possible underlying mech-anisms (24). Therefore, in the present study, we investigated the effect of hyperinsulinemia on the adrenaline-induced lipolysis during the different phases of the diet. The adrenaline-induced lipolysis during HEC was lower than that before initiation of HEC at all of the time points of the diet, which suggests that the interaction between insulin and adrenaline in the lipolysis control is not affected by the diet.

It is to be taken into account that the above-mentioned results are related to the abdominal SCAT and that the adap-tations in visceral or femoral/gluteal adipose tissue may be different. In in vivo studies, it was shown that the antilipolytic action of insulin (9) and β-adrenergic stimulation of lipolysis (8) were different in splanchnic vs. non-splanchnic and in upper body vs. lower body fat depots. With respect to the diet-induced changes, Maurière et al. (18) showed a different response of the adrenergic regulation of lipolysis in isolated adipocytes from the abdominal vs. femoral SCAT.

In conclusion, this study demonstrates that the dietary intervention associated with an improvement in insulin sensitivity modifies the adrenergic regulation of lipolysis in situ and that the pattern of the diet-induced modification is related to the duration of the diet and the status of energy balance. The lipid mobilization promoted by the physiological catecholamine adrenaline was enhanced because of a decrease in α2-adrenergic responsiveness during the dynamic phase of weight loss and returned back to the prediet levels during the subsequent WM phase. Thus, the major diet-induced changes in lipolysis were related to changes in α2-adrenergic regulation, whereas β-adrenergic and insulin-mediated regulation did not play a major role.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

K.K., M.S.-V., M.B., D.L., and V.S. did the conception and design of the research; K.K., J.P., M.B., I.d.G., and V.S. performed the experiments; K.K., M.S.-V., E.K., and M.A.M. analyzed the data; K.K., E.K., J.B., D.L., I.d.G., and V.S. interpreted the results of the experiments; K.K. drafted the manuscript; K.K., M.S.-V., J.B., D.L., I.d.G., and V.S. edited and revised the manuscript; K.K., M.S.-V., E.K., M.A.M., M.B., J.V.D.V., J.B., I.d.G., and V.S. approved the final version of the manuscript.

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