Modulation of triglyceride metabolism by glucocorticoids in diet-induced obesity

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Mantha, Line, Elena Palacios, and Yves Deshaies. Modulation of triglyceride metabolism by glucocorticoids in diet-induced obesity. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R455–R464, 1999.—The involvement of glucocorticoids (GC) in the development of diet-induced obesity and in the concomitant adaptations of triglyceride (TG)-rich lipoprotein metabolism were examined. Rats were fed either rodent chow, which maintains a low lipid flux, or a diet high in sucrose and fat (HSF) that increases lipid flux, leading to metabolic perturbations similar to those that define the plurimetabolic syndrome in humans. The GC status was manipulated through adrenalectomy (ADX) and corticosterone (Cort) replacement. Compared with chow, the HSF diet increased energy intake (17%) and whole body (8%) and adipose tissue (80%) weights. The HSF diet also increased the acute postprandial rise in plasma insulin (4-fold) and TG (3-fold), fasting liver TG content (3-fold), triglyceridemia (54%), and adipose tissue lipoprotein lipase (LPL) activity (2-fold). ADX decreased energy intake and whole body and adipose tissue weights in both dietary cohorts, but more so in HSF-fed than in chow-fed animals. These ADX-induced effects were totally prevented by Cort replacement in rats fed chow, but only partially so in those fed the HSF diet in proportion to the degree of restoration of energy intake. In the chow-fed cohort, the above indexes of TG metabolism remained unaffected by the Cort status, whereas in the HSF-fed cohort, these variables were decreased by ADX to levels of chow-fed animals. Cort replacement in the HSF-fed animals restored indexes of TG metabolism to intact levels and reestablished the diet-related differences observed in intact animals. These findings indicate that GC modulate fasting TG metabolism only minimally when a diet that maintains a low lipid flux is fed. In contrast, their presence is a necessary condition for the development of diet-induced obesity and the concomitant alterations in insulin sensitivity and TG-rich lipoprotein metabolism.

corticosterone; hepatic triglyceride secretion; insulin; lipoprotein lipase; food intake

Obesity is often accompanied by a cluster of metabolic abnormalities termed the plurimetabolic syndrome, or syndrome X (20). An abnormal lipoprotein profile constitutes one of the major metabolic abnormalities that confer to syndrome X its atherogenic potential. The dyslipidemia typical of the syndrome is associated with perturbations in the metabolism of triglyceride (TG)-rich lipoproteins (28, 44). Dietary factors are likely to be of importance in the etiology of syndrome X in humans. This is consistent with the fact that several of the risk factors associated with syndrome X, such as dyslipidemia and insulin resistance, can be induced in rodents by the consumption of diets high in carbohydrates such as fructose or sucrose (29, 37) or fats, particularly saturated fats (12, 47).

Deterioration of the lipoprotein profile in diet-induced obesity results partly from the excess intake of lipids and lipid precursors, but also from an increase in liver lipogenic activity due to a high intake of carbohydrates (14, 46). Excess exogenous and endogenous TG saturate the intravascular hydrolytic capacity of lipoprotein lipase (LPL) toward TG, which contributes to hypertriglyceridemia. Therefore, both determinants of the intravascular transport of lipids (absorption/synthesis and hydrolysis) contribute to the alterations in the lipoprotein profile brought by an energy-rich diet. The factors, other than nutrients themselves, that facilitate the necessary metabolic adaptations of lipid metabolism to accommodate such an increased lipid flux are incompletely understood.

Adrenal glucocorticoids (GC) are important modulators of energy balance. Removal of GC by adrenalectomy (ADX) attenuates or prevents the development of obesity in the vast majority of genetic and experimental models of obesity (45). The effects of ADX in obese rodents are reversed by exogenous administration of GC, thus highlighting the fundamental role of GC in the development of obesity (26). GC act at both the central level, where they affect neuronal pathways involved in the regulation of food intake and energy expenditure, and the periphery, where they modulate metabolic pathways that are liable to accommodate changes in energy balance (48). The levels at which GC may potentially influence the peripheral adaptations to an increased lipid flux are known (12, 13), but the contribution of GC to such peripheral adaptations of lipid metabolism remains to be determined.

The present study was therefore designed to test the hypothesis that GC are necessary for the development of diet-induced obesity and the related alterations in TG-rich lipoprotein metabolism. To this end, rats were fed either a diet that maintains a low lipid flux (rodent chow) or a diet that enhances lipid flux and leads to increased fat accretion and to a dyslipidemic profile resembling that of syndrome X. The GC status was manipulated by ADX, with or without Cort replacement. To gain insight into the metabolic pathways of TG metabolism that are affected by GC, hepatic TG content and plasma levels of TG were evaluated as indicators of endogenous TG production and transport, respectively. An assessment was further made of changes in hepatic TG secretion as well as key determinants of intravascular TG hydrolysis and subsequent
fatty acid uptake by tissues, namely postheparin plasma, adipose, and muscle LPL activity. Glycemia and insulinemia were monitored because of the central role played by insulin in both the production/secretion of endogenous TG and their intravascular hydrolysis by LPL and because GC modulate insulin secretion and efficiency of action.

MATERIALS AND METHODS

Animals and treatments. A first cohort of animals was used to determine the postprandial response of plasma glucose, insulin, and triglycerides to the diets. Twenty-four male Sprague-Dawley rats (Charles River, St. Constant, Quebec, Canada) initially weighing 225–250 g were housed individually in stainless steel cages in a room maintained at 24 ± 1°C, with a 12:12-h light-dark cycle (lights on at 2000). The animals were cared for and handled in compliance with the Canadian Guide for the Care and Use of Laboratory Animals, and the experimental procedures were approved by our institutional animal care committee. Rats were divided into two dietary groups of 12 animals each and for 2 wk had ad libitum access to water and either one of two diets. The first diet was a commercial, nonpurified diet (Charles River rodent chow #5075, Charles River, St. Constant, Quebec, Canada) with a gross energy content of 14.4 kJ/g, which maintains low levels of plasma lipids in the rat. The second diet consisted of a purified diet that leads to obesity, hyperlipidemia, and insulin resistance (22). The composition of the diet was the following: 41% of energy as carbohydrate (sucrose), 39% as lipid (corn oil:lard, 1:1), 20% as protein (casein), DL-methionine (0.3% wt/wt), vitamins (1.2%, vitamin mix no. 40060, Teklad Test Diets, Madison, WI), minerals (5.5%, AIN-76 mineral mix, ICN Biochemicals, Montreal, Quebec, Canada), and fiber (5.5%, Alphacel, ICN Biochemicals), with a gross energy content of 19.4 kJ/g. The purified diet, termed the high-sucrose, -fat (HSF) diet herein, was in the form of a paste and was provided to the animals in stainless steel, wire mesh-covered feeders attached to the inside of the cages. The nonpurified, pelleted diet was ground to a powder and provided in a similar manner. During the experimental period body weight and food intake were recorded every other day. To accurately monitor postprandial changes in the variables of interest, rats were subjected to the following meal intake protocol during the third week of treatment. At the beginning of the third week, access to food was restricted to the dark period. The animals were adapted during a total of 5 days to eat a meal within a period of 30 min 1 h after the onset of the dark period. The amount of food was not restricted during meal intake, and the animals typically ingested 5–8 g of food during the 30-min period. Free access to food was restored 90 min after the beginning of the meal, except on the day of the experiment. The meal intake protocol did not alter cumulative daily energy intake compared with ad libitum feeding. Two days into the meal adaptation period, the animals were fitted under isoflurane anesthesia with a permanent polyethylene cannula in the right jugular vein under isoflurane anesthesia. On the same day, the animals were subcutaneously implanted with a second slow-release pellet, with or without Cort, to maintain relatively constant levels of Cort in the blood throughout the experiment. Rats were treated for a total of 12 days. Food intake and body weight were measured every other day throughout the experimental period. The first cohort of animals was used to determine in vivo the rate of hepatic very low density lipoprotein-TG (VLDL-TG) secretion. Postheparin plasma and tissue LPL activities as well as plasma variables were determined in the second cohort. The postheparin plasma LPL procedure and tissue harvesting were separated by a 4-day period. The animals were fasted for 12 h during the lighted period before the procedures described below were performed so as to avoid the strong acute effects on lipid metabolism of the nutritional status, which was expected to differ between intact and ADX animals fed ad libitum.

VLDL-TG secretion rate. An initial blood sample (0.15 ml) was withdrawn through the venous catheter, and rats were injected through the catheter with 300 mg/kg body wt of Triton WR-1339 (Sigma, St. Louis, MO), a detergent that prevents intravascular TG catabolism (35). Blood samples (0.15 ml) were then taken 20, 40, and 60 min after the Triton injection. The rate of VLDL-TG secretion into the circulation was determined from regression analysis of TG accumulation in plasma versus time. Secretion rate was calculated by multiplying the slope of the regression line by plasma volume estimated from body weight and was expressed as micromoles per minute.

Postheparin plasma LPL. Approximately 0.5 ml of blood was drawn from the jugular catheter 10 min before and 10 min after the rapid intrajugular administration of 200 IU/kg body wt of sodium heparin (porcine intestinal mucosa, 1,000 USP/ml, Sigma) to release LPL from the vascular endothelium (33). Blood was centrifuged at 1,500 g, 4°C, for 15 min, and plasma was stored at –70°C for later measurement of postheparin plasma LPL activity.

Blood and tissue harvesting. Rats were killed by decapitation in the fasted state. Blood collected from the neck wound was centrifuged at 1,500 g, 4°C, for 15 min. Plasma was stored at –70°C for later biochemical measurements. A sample of liver was immediately frozen and stored at –70°C until later determination of TG content. Epididymal white
adipose tissue (WAT) and red vastus lateralis muscle (VLM) were excised. Tissues were weighed, ~50 mg were taken from WAT and the red portion of VLM, and tissue samples were homogenized using all-glass tissue grinders (Kontes, Vineland, NJ). The WAT samples were homogenized in 1 ml of a solution containing 0.25 M sucrose, 1 mM EDTA, 10 mM Tris·HCl, and 12 mM deoxycholate, pH 7.4. The VLM samples were homogenized in 1 ml of a solution containing 1 M ethylene glycol, 50 mM Tris·HCl, 3 mM deoxycholate, 10 IU/ml heparin, and 5% (vol/vol) aprotinin (Trasylol, Miles Pharmaceuticals, Rexdale, Ontario, Canada), pH 7.4. These homogenizing media were found to yield optimal LPL activities in the individual tissues. Homogenates of VLM were quickly frozen and stored at −70°C until measurement of LPL activity. WAT homogenates were centrifuged at 12,000 g, 4°C, for 20 min. The fraction between the upper fat layer and the bottom sediment was removed after tube slicing, diluted with 4 vol of the homogenization solution without deoxycholate, and stored at −70°C until later measurement of LPL activity.

Plasma variables. Insulin was quantitated by radioimmunoassay using a reagent kit from Linco Research (St. Charles, MO) with rat insulin as standard. Plasma corticosterone was determined by a competitive protein-binding assay (specificity: 0.058 nmol/L; interassay coefficient of variation: 9.0%) using plasma from a dexamethasone-treated female Rhesus monkey as a source of transcortin (36). Plasma glucose was determined with the use of a Beckman glucose analyzer (Beckman, Palo Alto, CA). Plasma TG concentrations were assayed by an enzymatic method with the use of a reagent kit from Boehringer Mannheim (Montreal, Quebec, Canada), which allows correction for free glycerol. Plasma nonesterified fatty acid (NEFA) concentrations were determined enzymatically with a reagent kit from Wako Pure Chemical Industries (Richmond, VA).

Lever TG content. Frozen liver samples were thawed and total lipids were extracted according to the method of Folch et al. (25) and solubilized in isopropanol. Triglycerides in the lipid extracts were then quantitated using the above-mentioned reagent kit.

Postheparin plasma and tissue LPL activities. LPL activity was measured in postheparin plasma and tissue homogenates as described earlier (41). Samples of 100 μl of postheparin plasma diluted 1:50 with saline and of WAT and VLM homogenates were incubated for 1 h at 28°C under gentle agitation with 100 μL of a substrate mixture consisting of a 0.2 M Tris·HCl buffer, pH 8.6, which contained 10 MBq/l carboxyl-[14C]triolein and 2.52 mM cold triolein emulsified in 5% gum arabic as well as 2% fatty-acid free bovine serum albumin, 10% human serum as a source of apolipoprotein C-II, and either 0.2 or 2 M NaCl. Free oleate released by LPL was then separated from intact triolein with chloroform-methanol and heptane (6) and mixed with Universol (New England Nuclear, Montreal, Quebec, Canada). Sample radioactivity was determined in an LKB Rackbeta liquid scintillation counter. LPL activity was calculated by subtracting non-LPL lipolytic activity determined in a final NaCl concentration of 1 M from total lipolytic activity determined in a final NaCl concentration of 0.1 M. In the present conditions, 1 M NaCl inhibited 82–91% of total lipolytic activity in all tissue homogenates and 30–40% of lipolytic activity (the remainder representing hepatic TG lipase) in postheparin plasma. Tissue LPL activity was expressed as microunits (1 μU = 1 μmol NEFA released per hour of incubation at 28°C) and plasma LPL activity as microunits per milliliter plasma. The inter assay coefficient of variation was 4.8% and was determined using bovine skim milk as a standard source of LPL.

Protein content of the tissue extracts was determined by the method of Lowry et al. (35) to calculate LPL specific activity (per unit protein). Total (per tissue) and specific activities were highly correlated (r = 0.92, P < 0.0001), and treatment effects were identical whether LPL was expressed as total or specific activity. Only total LPL activity is presented below.

Statistical analysis. Data are expressed as means ± SE. Factorial analysis of variance with repeated measures was performed to compare the acute postprandial response of glucose, insulin, and triglycerides to the two diets (cohort 1), the between-group factor being diet with two levels (nonpurified diet and HSF diet), and time after meal intake being the factor with repeated measures. A two-tailed Student’s t-test was used to compare means of the two dietary groups at the 0 (fasting) time point. Main treatment effects of diet and Cort status and treatment interactions were analyzed using factorial analysis of variance (cohorts 2 and 3), the factors being diet with two levels (nonpurified diet and HSF diet) and Cort status with three levels (intact, ADX, and ADX+Cort). In some instances, the intact and ADX groups were compared using a 2 × 2 factorial design to reveal treatment interactions without diluting the effect of the Cort status by the ADX+Cort group, which was expected to be comparable to the intact group. Individual between-group comparisons were performed using Fisher’s protected least squares difference post hoc test. Data were log transformed before analysis when group variances were not homogeneous (O’Brien’s test), but untransformed values are presented below. Pearson’s linear correlation coefficients were calculated to determine statistical associations between variables. Differences were considered statistically significant at P < 0.05.

RESULTS

The postprandial response of plasma glucose, insulin, and TG to the intake of a meal consisting of the habitual diet is shown in Fig. 1. Fasting plasma glucose was slightly (6%) but significantly (P < 0.04) higher in the HSF than in the nonpurified group (Fig. 1A). Both diets elicited the same postprandial rise in plasma glucose (main effect of time, P < 0.0001), and the incremental area under the glucose curve (not shown) did not differ among groups. The animals fed the HSF diet displayed fasting hyperinsulinemia (+284%, P < 0.02) compared with rats fed the nonpurified chow diet. Peak postprandial insulinemia was reached 30 min after the onset of the meal in both dietary groups, at which time it reached 0.50 and 1.84 nmol/l in the chow-fed and HSF-fed groups, respectively (Fig. 1B). Insulinemia remained higher in the HSF than in the chow group throughout the 6-h postprandial period, as witnessed by a significant treatment interaction [diet (D) × time (T) interaction, P < 0.04]. The incremental area under the insulin curve (not shown) was fourfold larger in the HSF than in the chow group. The slight difference between fasting plasma TG did not reach statistical significance (Fig. 1C). However, the postprandial rise in triglyceridemia, which peaked at 1–2 h after the onset of the meal, was significantly larger in the HSF than in the chow group throughout the period studied (D × T, P < 0.0008). The incremental area under the triglyceride curve (not shown) was 3.4-fold larger in the HSF than in the chow group.

ADX animals subcutaneously implanted with cholesterol pellets containing no Cort had undetectable levels.
of Cort in their blood (Fig. 2A). The implantation of Cort-containing pellets in ADX rats increased plasma Cort levels up to 0.1 µmol/l in both dietary cohorts, which were lower than in intact animals. Cort concentrations were significantly higher in intact rats fed the HSF diet than those fed chow [D × Cort status (C) interaction, P < 0.004], but they were unaffected by diet in ADX+Cort animals.

Diet and Cort status interacted on daily energy intake (D × C, P < 0.05), as depicted in Fig. 2B. The interaction was due to the fact that intact animals fed the HSF diet ingested 17% more energy than their counterparts fed the nonpurified diet, whereas both ADX and Cort replacement in ADX animals brought about similar energy intakes in the two dietary cohorts. ADX significantly reduced daily caloric intake in both dietary cohorts, but more so in HSF-fed (30% or −110 kJ/day) than in chow-fed rats (21% or −65 kJ/day), to levels that were identical in the two dietary cohorts. Cort replacement in ADX animals prevented the effects of ADX on energy intake in a diet-dependent fashion (D × C, P < 0.05), inasmuch as prevention was total in chow-fed rats but only partial in HSF-fed rats. Cort replacement maintained energy intake at identical levels in the two dietary groups. As expected, final body weight correlated with energy intake (r = 0.68, P < 0.0001). The treatments also interacted significantly.
(D × C, P < 0.05) on final body weight (Fig. 2C). The interaction stemmed from the following factors. Body weight was higher in intact (P < 0.03), but not in ADX or ADX + Cort rats fed the HSF diet compared with those fed chow; Cort replacement prevented the ADX-induced weight loss in chow-fed animals, but failed to do so in rats fed the HSF diet. ADX lowered body weight (main effect of C, P < 0.0004) in both chow-fed (−24 g) and HSF-fed (−48 g) animals. The treatment effects on energy intake and body weight described above were confirmed in the two consecutive studies.

Hepatic TG content was quantitated as an index of long-term liver triglyceride synthetic activity. The HSF diet increased liver TG content almost threefold (Fig. 3A) compared with the nonpurified diet. Diet strongly interacted with the Cort status on liver TG content (D × C, P = 0.0006). In the chow-fed cohort, although ADX tended to reduce liver TG (−0.036 mmol/g) and this was reversed by Cort replacement, post hoc analysis revealed that these effects were not significant. In contrast, the Cort status strongly influenced liver TG content in the HSF-fed cohort. ADX abolished the increase in hepatic TG associated with the intake of the HSF diet (−0.158 mmol/g, a reduction of 61%), and Cort replacement restored TG content to that of the intact group. Figure 3B shows that the HSF diet resulted in mild fasting hypertriglyceridemia compared with chow. Treatments interacted on triglyceridemia (D × C, P < 0.04), inasmuch as the Cort status had no impact in chow-fed animals but did interact in HSF-fed rats. In the latter, ADX tended to lower plasma triglyceride levels, which were returned to intact levels with Cort replacement. Figure 3C shows that TG secretion rate was altered according to the Cort status (main effect of C, P < 0.002), whereas diet had no overall effect. ADX decreased TG secretion rate slightly in chow-fed rats and by 32% in HSF-fed animals, whereas ADX + Cort groups had secretion rates comparable to those of intact animals. Whereas hepatic TG secretion rate and triglyceridemia evaluated in the same animals were modestly associated with each other (r = 0.43, P < 0.004) when all groups were considered together, correlation analysis according to diet revealed that the two variables were not related in chow-fed rats (r = 0.02) but were significantly related in HSF-fed animals (r = 0.58, P < 0.002).

Plasma levels of insulin were determined at the end of the experimental period, because the hormone is affected by the Cort status and obesity and because it constitutes an important comodulator of lipid metabolism. Figure 4A shows that the HSF diet caused hyperinsulinemia (main effect of D, P < 0.0001). ADX greatly lowered insulin concentrations in both chow (−73%) and HSF-fed (−81%) groups, but the absolute decrease was almost twofold larger in the HSF-fed animals (−0.186 nmol/l) than in those fed chow (−0.106 nmol/l), as witnessed by the significant treatment interaction (P < 0.003). In fact, removal of Cort through ADX in HSF-fed animals brought their insulin levels to those of ADX animals fed chow and therefore prevented diet-induced hyperinsulinemia. Cort replacement restored insulinemia to the intact range in both dietary cohorts, without any diet-related difference in the levels attained. Glycemia (Fig. 4B) was slightly but significantly affected by the Cort status (main effect of C, P < 0.0001). Plasma glucose levels were reduced in the ADX animals compared with intact and ADX + Cort groups. Post hoc analysis revealed that intact rats fed the HSF diet were hyperglycemic relative to their chow-fed counterparts, but that ADX + Cort groups fed either diet were identical to each other and to the intact chow-fed group. Plasma concentrations of NEFA, which

![Fig. 3. Liver triglyceride content (A), plasma triglyceride concentrations (B), and rates of hepatic triglyceride secretion (C) in sham-operated, ADX, and Cort rats. Bars represent means ± SE of 6–8 animals. Bars not sharing same superscript are different from each other at P < 0.05.](http://ajpregu.physiology.org/DownloadedFrom by 10.220.33.3 on October 30, 2017)
partly determine rates of hepatic TG synthesis and secretion, were found to remain unaltered by diet or the Cort status (data not shown). Figure 5 indicates that fasting postheparin plasma LPL, another major determinant of triglyceridemia, also remained unaffected by treatments. Both diet and the Cort status exerted strong actions on adipose tissue weight, and despite the lack of change in the global availability of LPL in the intravascular compartment, the treatments exerted major, tissue-specific actions on LPL activity (Fig. 5). Diet and the Cort status exerted significant main effects (P < 0.0001 and 0.0003, respectively) on retroperitoneal adipose weight without interacting with each other. Intact animals fed the HSF diet had 80% more retroperitoneal fat mass than their counterparts fed the nonpurified diet (Fig. 5A). ADX decreased adipose weight by 60% in both chow-fed and HSF-fed rats, which represented an absolute reduction of 0.63 and 1.10 g of fat, respectively. Cort replacement in ADX animals restored retroperitoneal weight toward intact values, but somewhat dampened the diet-related difference in tissue weight. Similar treatment effects were noted in the epididymal and inguinal adipose depots, as well as in the sum of the three depots (data not shown). As to retroperitoneal adipose LPL activity (Fig. 5B), diet and the Cort status also exerted significant main effects (P < 0.0003 and 0.001), and the Cort status tended to have stronger effects in HSF-fed than in chow-fed rats, as indicated by a nearly significant treatment interaction (P = 0.07). Indeed, post hoc analysis revealed that ADX resulted in a small (−4.6 µU/tissue) but nonsignificant reduction in adipose LPL in chow-fed rats, whereas the reduction reached 68% (−17.6 µU/tissue) in the HSF cohort. As in the case of adipose weight, Cort replacement restored LPL activity to levels that were not significantly different from those of intact animals, as well as the diet-related difference observed in intact animals. Adipose weight and LPL activity were significantly associated with each other (r = 0.65, P < 0.0007). There was no significant main effect of the diet on muscle weight. Cort replacement restored muscle weight toward that of intact rats, although in the HSF-fed cohort muscle weight remained significantly below intact values despite Cort treatment. The HSF diet reduced muscle LPL activity by an average of 42% (P < 0.003) relative to the chow cohort (Fig. 5D), whereas the Cort status did not alter enzyme activity in this skeletal muscle.

There were significant statistical associations between energy intake and most variables of TG metabolism that were evaluated in the same animals, including hepatic TG content (r = 0.70, P < 0.0001), triglyceridemia (r = 0.50, P < 0.0005), adipose tissue mass (r = 0.85, P < 0.0001), and LPL activity (r = 0.62, P < 0.0001). Insulinemia was in turn correlated with energy intake (r = 0.66, P < 0.0001) as well as with most variables of TG metabolism mentioned above (0.51 < r < 0.55, P < 0.0004).

**DISCUSSION**

This study aimed to assess the contribution of Cort to the adaptations of TG-rich lipoprotein metabolism and lipid deposition associated with diet-induced obesity. The findings demonstrate that GC modulated fasting TG metabolism only minimally in rats fed chow, a diet that maintains a low lipid flux. In contrast, GC were necessary for the stimulation of hepatic TG production and hypertriglyceridemia and for the adipose-specific increase in LPL in response to the HSF diet. Removal of these effects are exemplified by the nearly significant treatment interaction in Figure 5A and B, which reflects the relatively minor diet-related difference in adipose weight and LPL activity observed in intact animals. The results further illustrate that GC were necessary for the maintenance of normal adipose weight and LPL activity in rats fed chow, whereas the HSF diet elicited substantial differences in adipose weight and LPL activity. Cort replacement in ADX animals restored adipose weight and LPL activity toward intact values, but somewhat dampened the diet-related difference in tissue weight. The results also indicate that Cort replacement restored LPL activity to levels that were not significantly different from those of intact animals, as well as the diet-related difference observed in intact animals. Adipose weight and LPL activity were significantly associated with each other (r = 0.65, P < 0.0007). There was no significant main effect of the diet on muscle weight. Cort replacement restored muscle weight toward that of intact rats, although in the HSF-fed cohort muscle weight remained significantly below intact values despite Cort treatment. The HSF diet reduced muscle LPL activity by an average of 42% (P < 0.003) relative to the chow cohort (Fig. 5D), whereas the Cort status did not alter enzyme activity in this skeletal muscle.

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Cort abolished the diet-related differences in most determinants of TG metabolism and deposition, whereas these differences tended to be reestablished by Cort replacement. These findings indicate that Cort is among the necessary factors that facilitate lipid production and deposition in response to an obesity-promoting diet.

The HSF diet was particularly efficient in increasing lipid flux, confirming earlier studies (22, 30), because of both an increase in energy intake and the composition of the diet. After feeding the diet for 12 days, hepatic TG content, which reflects long-term endogenous TG production, was tripled in the HSF-fed intact animals, mainly because of the highly lipogenic potential of dietary sucrose (19). Fasting triglyceridemia was also slightly increased by the HSF diet, an effect that was dependent on the carbohydrate, rather than the fat content of the diet, as shown earlier (8). The rate of hepatic TG secretion measured after a 12-h fast was not affected by diet to a large extent. As shown by us and others (5, 19, 31), sucrose increases fasting hepatic TG secretion compared with a starch-based diet, but the presence of a high amount of fat in the diet tends to counteract this increase (8). The full impact of the HSF diet on triglyceridemia was also slightly increased by the HSF diet, an effect that was dependent on the carbohydrate, rather than the fat content of the diet, as shown earlier (8). The rate of hepatic TG secretion measured after a 12-h fast was not affected by diet to a large extent. As shown by us and others (5, 19, 31), sucrose increases fasting hepatic TG secretion compared with a starch-based diet, but the presence of a high amount of fat in the diet tends to counteract this increase (8). The full impact of the HSF diet on triglyceridemia could be appreciated in the postprandial state, during which overt hypertriglyceridemia was maintained for at least 6 h after meal intake. This was obviously a consequence of the large influx of dietary TG, but also likely the result of the sucrose-induced stimulation of lipogenesis and VLDL-TG secretion. The HSF diet also led to increased lipid deposition, a consequence of both an increased energy intake and of the lower metabolic cost of converting dietary fat into body fat compared with dietary carbohydrates (24). Although fasting postheparin plasma LPL activity remained unaltered by diet, enzyme activity increased in adipose tissue and decreased in muscle in response to the HSF diet, in a manner consistent with the preferential routing of TG substrates toward storage tissues. Diet-induced alterations in insulin levels were likely involved in the tissue-specific adaptations of LPL activity (9, 42). Finally, the HSF diet elicited insulin resistance, as reflected by fasting and postprandial hyperinsulinemia in the presence of normal glycemia compared with the chow-fed animals. As in the case of triglyceridemia, HSF-induced hyperinsulinemia was particularly evident in the postprandial state. The fat component of the diet was mainly involved in the development of insulin resistance (32), with some contribution from sucrose (40). Therefore, the HSF-fed rat can be considered as a model of diet-induced metabolic perturbations that are quite similar to those that define the plurimetabolic syndrome, which is frequently associated with human obesity (44).

Removal of the adrenals decreased circulating Cort to undetectable levels, whereas the implantation of pellets containing 40 mg of Cort in ADX animals increased plasma Cort levels up to 0.1 µmol/l, which were below those of intact rats. Cort levels in intact animals were obtained at 0800 (beginning of dark period) and correspond to peak values of the circadian rhythm of Cort (16). Therefore, the difference between Cort levels of intact and ADX+Cort groups was smaller...
at other times of the day than at the time of sampling. On the other hand, diet affected plasma levels of Cort, inasmuch as intact rats fed the HSF diet had higher fasting plasma Cort concentrations than those fed chow, in agreement with a previous study by Tannenbaum et al. (49) with high-fat-fed rats.

The ADX-induced reduction in food intake, body weight, and fat mass confirms the well-established involvement of GC in energy balance (10, 17, 48, 52). Treatment of ADX animals with Cort restored most of the metabolic alterations brought about by ADX, indicating that removal of Cort was the principal causative factor in the effects of ADX. However, the degree of restoration of energy intake was diet dependent, confirming earlier findings (2). Indeed, whereas Cort treatment restored food intake to intact levels in chow-fed animals, the anorectic action of ADX was partially maintained in HSF-fed animals implanted with Cort. Body and tissue weights reflected these diet-related differences in the effects of Cort replacement on energy intake. The findings are in agreement with the previously reported dependence on diet composition of the magnitude of effect of GC receptor blockade on adipose mass (38). The reasons for the lack of full reversal of the effects of ADX on energy intake by Cort in HSF-fed animals are unknown. Indexes of insulin action were also affected by the Cort status in a diet-dependent manner. The influence of the Cort status was clearly more robust in the HSF-fed than in the chow-fed cohort, as indicated by the treatment interaction on insulinemia. Improvement of insulin sensitivity by ADX has been reported in other models of obesity (7, 26). Cort replacement prevented the effects of ADX on glycemia and insulinemia in proportion to its action on energy intake, that is, a full and partial reversal in chow- and HSF-fed animals, respectively.

Removal of GC through ADX had a major impact on all determinants of TG metabolism that was strongly diet dependent. Indeed, although weak, nonsignificant trends were noted in the chow-fed cohort, variables of TG metabolism remained essentially unaffected by the Cort status, including liver TG content, hepatic TG secretion rate, triglyceridemia, and adipose tissue LPL activity. This lack of effect of the Cort status on fasting TG metabolism in chow-fed rats occurred despite fluctuations of as much as 20% in energy intake. Therefore, Cort does not appear to modulate TG metabolism to any significant extent when lipid flux tends to be low, such as when a nonpurified, low-fat diet high in complex carbohydrates is fed. In sharp contrast, Cort proved to be essential in the establishment of diet-related differences in indexes of lipid metabolism, and all were greatly reduced by ADX in animals fed the HSF diet. In fact, in HSF-fed ADX animals, liver TG content, hepatic TG secretion rate, triglyceridemia, and adipose tissue LPL activity became indistinguishable from those of their chow-fed counterparts, despite the high lipogenic potential of the constituent macronutrients of the HSF diet. The one exception to this was muscle LPL, which remained lower in HSF-fed than in chow-fed animals regardless of the Cort status. Muscle LPL may be particularly sensitive to lipid flux and oxidation (23), and the absence of effect of the Cort status on muscle LPL confirms earlier findings (18).

As was the case for ADX itself, Cort replacement did not significantly affect determinants of TG metabolism in the chow-fed cohort. In contrast, liver TG content, triglyceridemia, hepatic TG secretion, and adipose tissue LPL of HSF-fed rats, which were all decreased by ADX, were fully restored to intact levels by Cort treatment of ADX animals. Moreover, most of the diet-related differences that were observed in intact animals were reestablished in Cort-implanted animals, despite the absence of hyperphagia in HSF-fed rats relative to their chow-fed counterparts. These findings indicate that determinants of TG metabolism are particularly sensitive to diet composition, because they were modulated by the nature of the diet even in the presence of equal caloric intake and similar plasma Cort concentration. However, Cort had to be present for such diet-related differences to be expressed, because they were no longer seen in ADX animals without Cort replacement. This may be related to the fact that pair feeding among the two dietary cohorts was achieved at lower intake levels in ADX rats compared with ADX+Cort animals.

The modes of action of GC on lipid metabolism are manifold. First, the centrally mediated anorectic and thermogenic effects of GC removal evidently decrease TG synthesis in the liver, their secretion and transport into the circulation, as well as their deposition into lipid stores. Second, GC exert several direct peripheral actions that sustain lipid production, transport, and storage, which would be liable to be downregulated after ADX. In vitro studies have shown that GC directly stimulate hepatic fatty acid and TG synthesis (11, 21) and increase apolipoprotein B secretion in hepatocytes (51), thereby favoring VLDL secretion into the circulation (4, 43). Although without much effect by themselves (1, 15), GC potentiate the positive modulation of adipose tissue LPL by insulin (3, 27, 39). Third, GC tend to stimulate insulin secretion through several mechanisms (7, 50). Insulin shares with Cort most of its actions on lipid production and storage, including the stimulation of hepatic lipogenesis and adipose LPL activity, with the exception of VLDL secretion by the insulin-sensitive liver (34). The respective contribution of central and peripheral actions of GC on overall lipid metabolism remains to be determined. In the present studies, there was a strong association between energy intake and most of the determinants of lipid production, transport, and deposition. In addition, basal, or threshold, levels of these determinants (e.g., hepatic TG production and secretion, adipose LPL activity) were maintained in total absence of Cort. These findings suggest that the peripheral actions of GC may serve as supportive adaptations to accommodate the central action of GC on ingestive behavior.

Perspectives

Sustained consumption of diets high in insulinogetic/lipogenic carbohydrates and in fat promotes obesity
and its metabolic complications. The deleterious effects of diet-induced obesity include alterations in the metabolism of lipid substrates. These are partly caused by complex endocrine adaptations that occur to adjust the organism to an elevated energy flux. The present studies underline the role of GC in these processes. As stated by Tannenbaum et al. (49), high-energy (particularly high fat) diets act as a background form of chronic stress that elevates basal GC levels. A vicious cycle is established, as high GC levels are likely to favor increased food intake through their action on the central regulatory pathways of energy balance. At the periphery, GC have the potential to directly facilitate lipid production from carbohydrates as well as deposition of triglycerides from endogenous and exogenous sources. In addition, high GC levels favor an increased insulin secretion. They also contribute to the development of insulin resistance of metabolic pathways that tend to decrease lipid flux (anorectic action, glucose use, inhibition of lipolysis, VLDL secretion), whereas pathways that increase lipid flux remain responsive to insulin (liver lipid production, deposition-promoting adipose lipoprotein lipase). GC themselves potentiate the action of insulin on several of these pathways. Concomitantly, endocrine factors that promote triglyceride mobilization and utilization, such as the sympathetic adrenal system, may become less active. The sum of these adaptations constitutes an exquisitely integrated response of the organism to increased energy availabil-

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