Effect of a High-Fat Diet on Food Intake and Hypothalamic Neuropeptide Gene Expression in Streptozotocin Diabetes

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Abstract

Insulin-deficient diabetic rats are markedly hyperphagic when fed a high-carbohydrate (HC) diet, but normophagic when fed a high-fat (HF) diet. When maintained on a HC diet, diabetic rats also exhibit increased gene expression of the orexigenic peptide neuropeptide Y (NPY) in the hypothalamic arcuate nucleus, and reduced expression of the anorectic peptide corticotropin-releasing hormone (CRH) in the paraventricular nucleus, and these changes are hypothesized to contribute to diabetic hyperphagia. In this experiment we assessed whether the normophagia displayed by HF-fed diabetic rats is associated with the opposite profile of NPY and CRH expression. Our results show that relative to diabetic rats on the HC diet, the diabetic rats on the HF diet exhibited significantly reduced caloric intake (−40%), NPY expression in the arcuate nucleus (−27%), and elevated CRH expression in the paraventricular nucleus (+37%). Insulin and corticosterone, which are known to affect hypothalamic NPY and CRH expression, were not different between these two groups, making it unlikely that they can account for the differences in either feeding behavior or hypothalamic peptide expression. There was a small but significant increase in plasma leptin levels in the diabetic animals maintained on the HF, and large differences in parameters associated with elevated fat oxidation. These observations support the hypothesis that the normalization of food intake observed in diabetic rats consuming a HF diet may in part be mediated by reductions in NPY expression and elevations in CRH expression. (*J. Clin. Invest.* 1998. 102:340–346.) Key words: neuropeptide Y • corticotropin-releasing hormone • high-fat diet • streptozotocin

Introduction

The hyperphagia of rats with uncontrolled insulin-dependent diabetes mellitus has been extensively studied for many years and has generated a number of hypotheses about the metabolic controls of food intake (1). Many of the metabolic disturbances associated with experimental diabetes have been suggested as the cause of diabetic hyperphagia, including impaired glucose utilization (2), reduced body adiposity (3), and low insulin concentrations (4–8). Other studies focusing on the role of diet in diabetic hyperphagia suggest that overeating is due to a lack of readily oxidizable fuels (9). Support for this latter hypothesis is based in part on the observation that food intake is normalized by feeding diabetic rats a high-fat (HF)[1] diet. More specifically, this hypothesis states that in the absence of insulin, fats are more readily oxidized than carbohydrates, and on the basis of their differential ability to extract energy from the two nutrient sources, diabetic rats alter food intake accordingly (10, 11). How the brain senses alterations in fuel oxidation and what central nervous system (CNS) mechanisms are involved in the changes in food intake in diabetic rats fed a diet high in fat are unknown.

Hypothalamic neuropeptide Y (NPY) and corticotropin-releasing hormone (CRH) are hypothesized to be involved in diabetic hyperphagia (7). After the chemical induction of diabetes, NPY mRNA levels in the hypothalamic arcuate nucleus (ARC) increase (5, 7), as does NPY content and release in the paraventricular nucleus (PVN) (4, 6, 12). Support for the argument that elevated hypothalamic NPYergic activity contributes to diabetic hyperphagia is based in part on the observations that in nondiabetic animals, NPY injection into the PVN stimulates food intake (13), and when repeatedly administered into this region produces both persistent hyperphagia and obesity (14). In diabetic rats on a high-carbohydrate (HC) diet, CRH expression in the PVN is reduced (7). This observation is consistent with diabetic hyperphagia in that acute CRH administration into the PVN reduces food intake (15), and chronic administration reduces both food intake and body weight (16, 17). Moreover, there is evidence that CRH acts in the PVN to directly oppose the stimulatory actions of hypothalamic NPY on feeding (18).

One hypothesis that explains the changes in both hypothalamic peptide systems and feeding behavior of diabetic rats fed a HC diet is based on changes in the levels of insulin and corticosterone in the CNS. Elevated insulin concentrations within the CNS are thought to reduce the production and release of NPY in the hypothalamus, as well as food intake (19). Conversely, in the diabetic state, reduced CNS insulin concentrations are proposed to result in the elevation of hypothalamic

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1. *Abbreviations used in this paper:* ARC, arcuate nucleus; CNS, central nervous system; CRH, corticotropin-releasing hormone; HC, high-carbohydrate; HF, high-fat; NPY, neuropeptide Y; PVN, paraventricular nucleus; STZ, streptozotocin.

NPY activity, which in turn produces hyperphagia (7). In contrast to reduced insulin levels, plasma corticosterone concentrations are elevated in diabetic relative to nondiabetic rats (20, 21), and this occurs despite the reduction in PVN CRH expression (7). Moreover, corticosterone administration to diabetic rats increases both ARC NPY expression and food intake in a dose-dependent manner (22).

To examine further the involvement of hypothalamic NPY and CRH in the feeding behavior of diabetic rats, we manipulated their food intake by feeding them diets either low or high in fat. In nondiabetic rats, HF diets can increase caloric intake, an effect opposite to that observed in diabetic animals. However, this effect is dependent on both the composition of the diet and the strain of rat. To eliminate a potentially confounding effect of the HF diet in the nondiabetic group, the composition of the diet (23) and strain of rat were selected specifically to have no effect on caloric intake in the nondiabetic animals. This permitted a more specific assessment of the impact of HF feeding on the hypothalamic responses to uncontrolled diabetes. This experimental manipulation provides a powerful model since many of the metabolic disturbances of diabetes persist in diabetic rats fed a HF diet despite the normalization of food intake (9, 11).

Using this model we sought to determine whether this HF feeding–induced normophagia in diabetic rats is associated with reduced NPY mRNA levels in the ARC, and/or increased CRH mRNA levels in the PVN, relative to diabetic rats on the more standard HC diet. If this profile of hypothalamic peptide expression is observed, it would suggest that the normophagia exhibited by diabetic rats on a HF diet may be mediated, at least in part, by opposing changes in NPY and CRH biosynthesis. In addition, it would assess whether changes in these hypothalamic systems can occur independently of differences in insulin and/or corticosterone, and would permit us to investigate other metabolic factors that may be driving peptide expression in the hypothalamus. Alternatively, if diet does not affect the hypothalamic expression of these two peptides, then it would indicate that these hypothalamic systems are not involved in the normophagia displayed by diabetic rats on the HF diet, and would further suggest that the combination of elevated hypothalamic NPY and reduced CRH biosynthesis does not necessarily produce hyperphagia. Therefore, we measured food intake, neuropeptide mRNA levels, circulating fuels, and plasma insulin, corticosterone, and leptin levels, and compared these to values measured in nondiabetic rats maintained on one of the two different diets.

Methods

Male Long-Evans rats weighing an average of 346±7.3 g were obtained from the breeding colony maintained by the Department of Psychology at the University of Washington. Rats were housed individually in a temperature-controlled environment (22°C) with a 12-h light/12-h dark cycle. All rats had ad libitum access to pelleted rat chow and tap water except where noted otherwise. The study protocol used in this experiment was approved by the Animal Care Committee at the University of Washington.

Experimental diabetes. Animals were separated into two groups of equivalent mean body weight and food intakes before streptozotocin (STZ) or vehicle treatment. Rats in one group (n = 30) were made diabetic while under light halothane anesthesia by tail vein injection of STZ (65 mg/kg) (Sigma Chemical Co., St. Louis, MO) diluted in 0.5 ml sterile citrate buffer. Rats in the second group (n = 16 in total) were injected with citrate buffer only and served as nondiabetic controls.

Table I. Composition of Experimental Diets

Ingredients (g/100 g)	HF/LC (HF)	HC/LF (HC)
Casein	20.0	20.0
Cornstarch	10.4	52.0
Corn oil	23.1	4.6
Alphacel	41.5	18.4
AIN mineral mix	3.5	3.5
AIN vitamin mix	1.0	1.0
DL-Methionine	0.3	0.3
Choline bitartrate	0.2	0.2
% kcal protein	24.0	24.0
% kcal fat	63.0	13.9
% kcal carbohydrate	13.0	63
kcal/g	3.3	3.3

AIN mixes are AIN 76 formulas (now AIN 93G).

Experimental protocol. After the STZ or vehicle treatments, the two groups of animals were maintained on their standard chow diet for 12 d with food intake and body weights monitored daily. At the end of this interval one group each of the surviving diabetic rats (n = 14) and one group of nondiabetic rats (n = 8) were placed on a HF diet, and the remaining two groups were placed on the HC diet (n = 13 diabetic, n = 7 nondiabetic). For diet composition see Table I. Food intake and body weights were again monitored daily for the next 12 d, after which animals were killed by decapitation. Trunk blood was collected and brains were rapidly removed and frozen using liquid freon. Blood was kept on ice until the plasma was separated and then stored at −75°C until assayed for insulin, glucose, corticosterone, leptin, triglycerides, free fatty acids, and ketone bodies.

Plasma assays. Plasma insulin was measured by RIA using a kit with a human insulin standard (Diagnostic Systems Lab, Webster, TX) with a slight modification (7). Corticosterone was measured using a previously described RIA (24). Plasma glucose was determined by the glucose oxidase method with a Beckman glucose autoanalyzer (Beckman Instruments, Inc., Brea, CA). FFA were measured enzymatically using a commercially available kit (Wako Chemicals, Richmond, VA). Triglycerides were assayed according to a modification of the enzymatic method described by Wieland (25). Ketone bodies (acetoacetate and β-hydroxybutyrate) were measured using a modification of the enzymatic procedure described by Bates et al. (26). Leptin levels were measured by RIA for mouse leptin using a commercially available kit (Linco, St. Louis, MO) (27). This mouse assay is quite comparable to the results that are obtained from the now available rat assays. The two assays are highly correlated (r = 0.94) and consequently while the absolute leptin levels reported by the two assays may differ slightly, it is clear that the mouse assay provides accurate information about the relative differences in leptin levels between the groups in this experiment (28).

In situ hybridization. Coronal sections of frozen rat brain were sectioned at 14 μm on a cryostat and mounted on RNase-free slides stored at −70°C. After tissue fixation, hybridization was performed using antisense oligonucleotide probes based on cDNA sequences of rat NPY or CRH genes (see reference 7 for details). The probes were labeled with [^{33}P]adenosine, and after hybridization slides they were rinsed under high-stringency conditions and opposed to x-ray film to generate autoradiographs, which were analyzed using computer densitometry. Using a standard curve, autoradiograph optical density and total hybridization area were determined using the MCID image analysis system (Imaging Research, St. Catherines, Ontario, Canada).

Measurements of NPY mRNA were made on six to eight sections per rat from the midregion of the rostral-caudal extent of the ARC selected by an investigator blind to the study conditions. Measurements for CRH used a similar approach on sections obtained from the PVN. Because of the number of animals and tissue sections involved in this study, the diabetic and nondiabetic groups were run in separate assays. All densitometry data were collected by a technician blind to the treatment group of the sections being analyzed. The product of hybridization area and density was used as an index of overall mRNA levels.

Statistics. Food intake and body weight data used for graphical presentation and statistical analysis are based on the last 3 d of each of the three experimental time periods (pre-STZ baseline, post-STZ period during which all animals were maintained on laboratory chow, and days 9–12 after the animals had been consuming either the HF or HC diets). Food intake and body weight data were analyzed by ANOVA with three factors, diet, diabetes, and time, with time treated as a repeated measure. Comparisons among individual means were made by Tukey's t test. Data derived from the plasma assays were analyzed using an ANOVA with two factors, diet and diabetes, followed by Bonferroni's t test. Because the in situ hybridization assays were done at separate times for the diabetic and nondiabetic animals, statistical comparisons of mRNA levels between these groups (diabetic vs. nondiabetic) cannot be made. Therefore, nonpaired, two-tailed t tests were used to analyze the effect of the diet on mRNA levels. In the text, table, and figures, all data are presented as means±SEM.

Results

Food intake for the three experimental time periods is depicted in Fig. 1. Baseline food intake was not significantly different among the four groups of animals before STZ administration ($F\{3,76\} = 0.18$, $P = 0.91$). After STZ administration and during the period when all rats were maintained on standard laboratory chow, diabetic rats had significantly increased food intake relative to nondiabetic controls (187.5±6.5 vs. 113±8 kcal) ($F\{1,76\} = 116.2$, $P < 0.01$). After the change from the chow diet to the experimental diets, the diabetic animals fed the HC diet continued to consume significantly more food than the other three groups ($F\{3,76\} = 52.4$, $P < 0.01$ for interaction). In contrast, total daily caloric intake of the diabetic animals on the HF diet did not differ significantly from either the HF- or HC-fed nondiabetic control groups, and consumption of the HF diet did not affect caloric intake in nondiabetic rats. These results are consistent with other reports using a similar protocol (9, 29, 30).

Body weights for the three experimental time periods are depicted in Fig. 2. Before STZ administration, there were no differences in body weight among the four groups of rats ($F\{3,76\} = 0.24$, $P = 0.86$). Subsequently, there was a significant decrease in body weight for the animals that received the STZ, relative to vehicle-treated controls ($F\{3,76\} = 5.2$, $P < 0.002$). This occurred during the period when all the animals were maintained on the standard chow diets. This difference in body weight between the STZ and vehicle-treated rats was maintained after the change to either the HF or HC diets. There was no significant difference in body weight between the diabetic groups fed the two different diets ($F\{2,76\} = 0.14$, $P < 0.91$), and as we expected, there was no difference in body weight between the nondiabetics fed the different diets ($F\{2,76\} = 0.16$, $P = 0.86$).

All plasma hormone and substrate levels were determined on samples obtained at the conclusion of the study and the data are presented in Table II. Whereas STZ-treated rats had significantly lower plasma insulin levels than controls ($F\{1,39\} = 127.0$, $P < 0.001$), there was no difference in plasma insulin levels between the two groups of diabetic animals on the two diets ($t\{26\} = -0.07$, $P = 0.41$). STZ-treated rats had higher plasma glucose levels than the nondiabetic animals ($F\{1,39\} = 12.7$, $P < 0.01$). Plasma glucose concentrations were significantly elevated in the diabetic group maintained on the HC diet relative to the HF-fed diabetic group ($t\{26\} = -3.5$, $P < 0.002$). There was no statistical difference in plasma glucose levels between the nondiabetic groups ($t\{9\} = 0.09$, $P = 0.46$).

Diabetic rats exhibited lower levels of plasma leptin relative to nondiabetic rats ($F\{1,39\} = 215.9$, $P < 0.0001$) (31). Interestingly, plasma leptin was significantly elevated in the diabetic rats on the HF diet, in comparison to the diabetic rats on the HC diet ($t\{27\} = 3.7$, $P < 0.001$). Diabetic animals had significantly elevated triglyceride levels relative to nondiabetics ($F\{1,38\} = 8.9$, $P < 0.008$). Individual comparisons revealed a significant increase in plasma triglyceride concentrations between the two diabetic groups, with the HF group having higher concentrations ($t\{24\} = 2.5$, $P < 0.02$). Rats maintained on the HF diet had elevated plasma FFA levels compared with those fed the HC diet ($F\{3,38\} = 15.3$, $P < 0.004$). There was

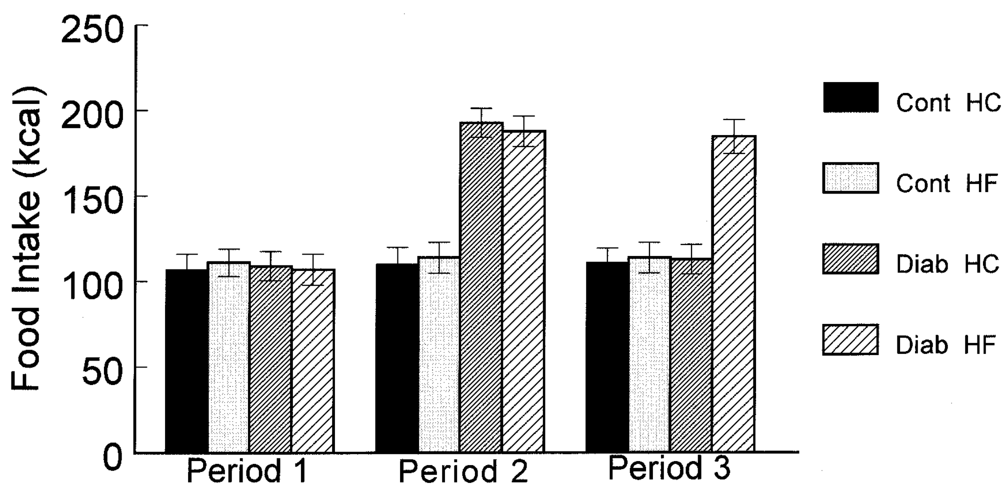

Figure 1. Mean (±SE) food intake for each of the four groups of rats averaged over the last 3 d of each experimental time period.

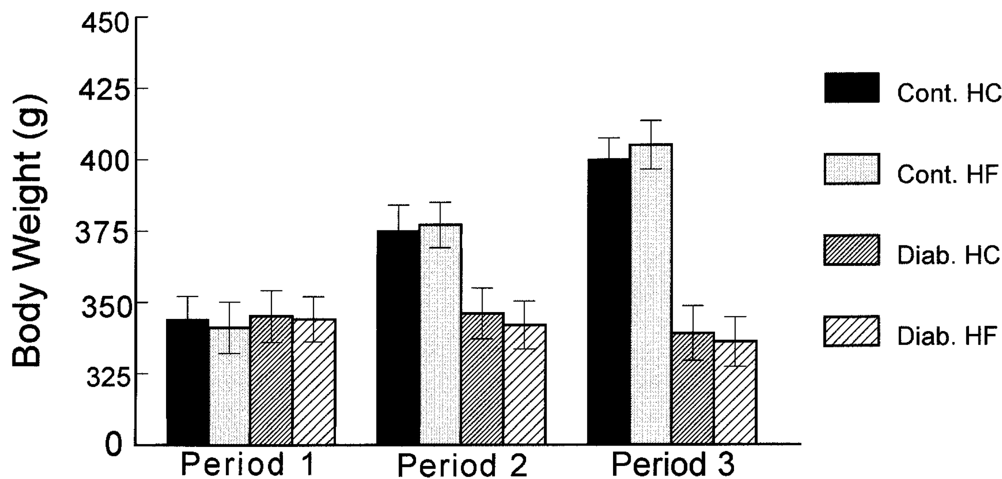

Figure 2. Mean (±SE) body weight for each of the four groups averaged over the last 3 d of each experimental time period.

also a significant difference in FFA between the diabetic groups, with the HF group having higher levels (t{23} = 3.54, $P < 0.01$), whereas the difference in FFA concentrations between the nondiabetic animals was not significant (t{12} = 2.2, $P < 0.08$). Plasma ketone body levels were significantly higher in the diabetic animals fed the HF diet than those of the other three groups (F{1,32} = 7.7, $P < 0.009$, for interaction).

Between the diabetic groups, NPY gene expression was significantly reduced in the HF-fed animals (t{22} = −2.8, $P < 0.009$). In these same two groups, CRH gene expression was elevated in the HF-fed group (t{16} = 3.04, $P < 0.007$) (Fig. 3). Consistent with the food intake and body weight data, there was no difference in either NPY (t{11} = 0.087, $P < 0.93$) or CRH gene expression (t{8} = −1.18, $P < 0.27$) between the two groups of nondiabetic animals (Fig. 4).

Discussion

Consistent with previous reports, diabetic animals maintained on a HC diet were hyperphagic relative to diabetic rats maintained on a HF diet, consuming 40% more calories on a daily basis (9, 29, 30). A new finding is that the diabetic group on the HF diet displayed significantly reduced ARC NPY expression (−27%) and elevated PVN CRH expression (+37%) relative to their diabetic counterparts on the HC diets. Since diabetes results in an elevation in hypothalamic NPY mRNA concentrations, and a reduction in CRH mRNA concentrations in HC-fed rats, the main effect of feeding the HF diet was therefore to attenuate the typical hypothalamic response to diabetes (7). In contrast, there was no difference in caloric intake, hypothalamic NPY expression, or CRH expression in nondiabetic rats fed the different diets. In a previous experiment, Giraudo et al. reported that nondiabetic rats fed a HF diet exhibited decreased NPY mRNA in the ARC (30). In that experiment, however, the HF diet was associated with increased caloric intake and weight gain, an effect not observed in our study. Since expansion of the adipose depot leads to increased secretion of hormones that inhibit hypothalamic NPY gene expression (e.g., leptin and insulin), differences in weight gain associated with different HF diets may explain the earlier results. While the experimental design did not permit direct comparisons of the level of neuropeptide mRNA between nondiabetic and diabetic groups, we conclude that differences in caloric intake resulting from feeding diabetic rats either a HF or HC diet are associated with changes of NPY and CRH mRNA concentrations. Therefore, these alterations in peptide expression may have contributed to the changes in feeding behavior. In the nondiabetic groups, there was no effect of diet on food intake, body weight, or hypothalamic peptide expression.

It is important to note that previous reports have shown a

Table II. Plasma Hormone and Metabolic Substrate Concentrations

	Diabetic rats		Nondiabetic rats	
Plasma measures	HF diet	HC diet	HF diet	HC diet
Insulin μU/ml	18.8±2.6* (13)	19.1±3.3* (13)	116.7±48.0‡ (7)	139.6±15.8‡ (7)
Glucose mg/dl	398.6±23.5* (13)	505.7±30.4‡ (13)	139.8±7.0§ (6)	138.5±5.1§ (6)
Corticosterone μg/dl	4.7±1.7* (13)	4.3±1.8* (13)	—	—
Leptin ng/ml	1.1±0.09* (13)	0.7±0.03‡ (12)	4.2±0.29§ (7)	3.2±1.3§
Triglycerides mmol	4.2±0.63* (14)	2.5±1.41‡ (13)	3.4±1.8*‡ (7)	2.4±0.29‡ (7)
FFA mmol	1.0±0.16* (14)	0.63±0.20‡ (13)	0.67±0.28‡ (6)	0.49±0.047‡ (7)
Ketone bodies mmol	4.8±0.61* (12)	1.6±0.41‡ (13)	0.47±0.23§ (6)	0.15±0.05§ (7)

All values are the mean±SE for the number of samples indicated in parentheses. Within a row, different symbols indicate significant differences of at least $P < 0.05$. The numbers in the parentheses are the sample size for that specific measure and group.

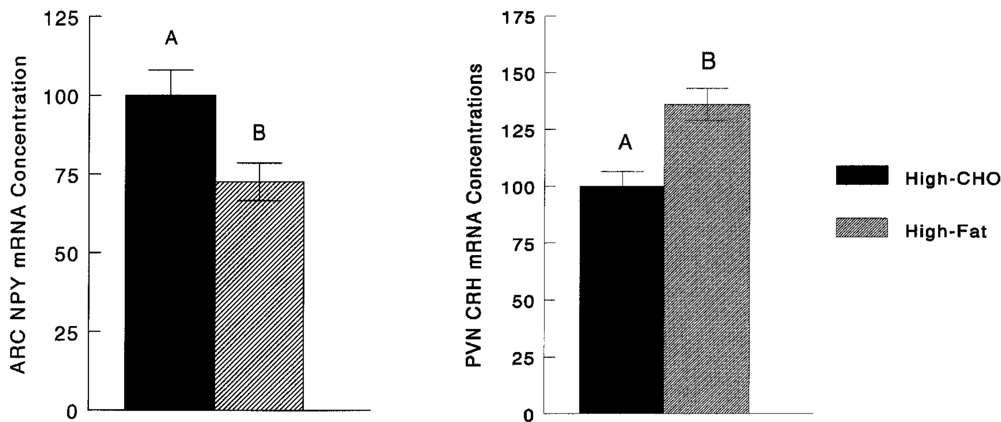

Figure 3. Mean (±SE) mRNA concentrations for NPY and CRH in the ARC and PVN for diabetic rats on either the HF or HC diets. The diabetic animals fed the HC diet serve as the controls. Values represent area × density measures derived from computer densitometry. The different letter superscripts indicate a significant difference of at least $P < 0.01$.

67–200% elevation in ARC NPY mRNA concentrations in diabetic rats compared with nondiabetic rats maintained on standard laboratory chow diets (5, 7). Thus, the modest decline in NPY mRNA concentrations observed in the HF-fed diabetic rats was unlikely to have normalized the increase in NPY gene expression that occurs in diabetes. Extrapolating from a previous report in which PVN CRH mRNA levels were reduced by 44% in diabetic relative to nondiabetic rats (7), CRH gene expression in our experiment may have been nearly normalized in the HF-fed diabetic group, relative to nondiabetic controls. Nonetheless, factors additional to NPY and CRH likely contributed to the effects of diet composition on food intake in diabetic rats and further studies are warranted to identify and characterize them.

There was no difference in plasma insulin or corticosterone levels, or body weight, between the two groups of diabetic animals. Thus, even though these two hormones are important in the control of NPY and CRH expression in the hypothalamus, it is unlikely that they alone can account for the differences observed in food intake or neuropeptide mRNA levels. These results further suggest that factors associated with the provision of fat fuels can also modulate hypothalamic peptide expression. However, before we can exclude the differences in corticosterone as contributing to the differences in peptide expression, studies examining the effect of diabetes and diet on circadian patterning of the hypothalamic-pituitary-adrenal axis are required. Nonetheless, our data and others (7, 32) raise the possibility of a dissociation between levels of plasma corticosterone and of PVN CRH gene expression. Specifically, the preservation of elevated corticosterone levels despite reduced CRH mRNA levels in the HC group of diabetic rats suggests the possibility that corticosterone elevation occurred via factors in addition to, or separate from, CRH.

The results of this experiment are consistent with the hypothesis that it is not the diet composition or hormone levels per se that altered hypothalamic peptide expression and feeding behavior, but rather is the differential ability of the diabetic rats to utilize fat and carbohydrate substrates (9, 11). Thus, fuel oxidation may be an independent signal that can affect hypothalamic NPY and CRH expression in a direction consistent with feeding behavior. This interpretation of our data suggests a possible hypothalamic mechanism by which fuel oxidation per se may alter caloric intake. However, the signal(s) by which the CNS might sense differences in the oxidation of fuels remains unclear. One possibility is that an oxidative signal is transmitted to the CNS via a neural circuit. There is evidence that hepatic sensors can detect various nutrients, metabolites, and the production of energy from oxidative processes, and that some of these signals are transmitted to the brain by visceral afferents to alter feeding behavior (33). If our results are based, at least in part, on a peripheral oxidative signal which is transmitted to the CNS by visceral afferents, then

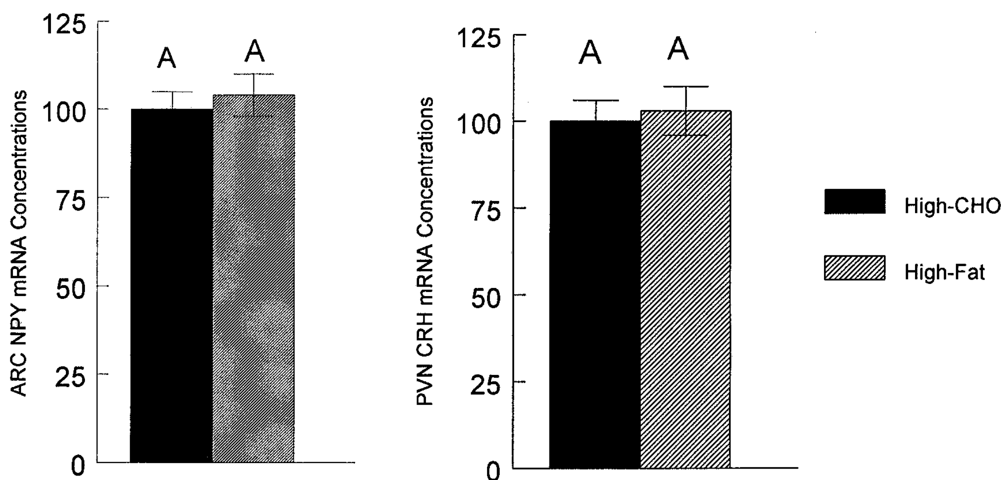

Figure 4. Mean (±SE) mRNA for NPY and CRH in the ARC and PVN for nondiabetic rats on either the HF or HC diet. The nondiabetic animals fed the HC diet serve as the controls. Values represent area × density measures derived from computer densitometry. The different letter superscripts indicate a significant difference of at least $P < 0.01$.

ablation of these nerves may to some extent attenuate the reduction in intake of HF diets, as well as peptide expression.

The recently identified hormone leptin, the product of the *ob* gene expressed only in adipose tissue (34), appears to regulate adiposity in part by reducing food intake (35–39). In food-deprived Long-Evans rats, leptin administration directly into the CNS reduces food intake, body weight, and NPY expression in the hypothalamus, while increasing CRH expression relative to vehicle-infused animals (40, 41). It is noteworthy that these effects are essentially opposite to those observed in uncontrolled diabetes, suggesting that leptin deficiency may contribute to the effect of diabetes on hypothalamic function. Moreover, STZ diabetes reduces adipocyte leptin mRNA levels in mice (30), and our data are in agreement with this report in that leptin levels were 76% lower in diabetic as compared with nondiabetics rats. In agreement with human data, feeding a HF diet had no effect on plasma leptin levels in the nondiabetic control rats (27). Diabetic rats fed the HF diet had significantly elevated plasma leptin levels relative to diabetic rats fed the HC diet (1.1 ± 0.09 vs. 0.7 ± 0.03 ng/ml, respectively). However, relative to the nondiabetic controls, both groups of diabetic rats showed an equivalent decrease in plasma leptin levels. Since the increase in plasma leptin concentrations of HF-fed diabetic rats was small and did not normalize the leptin levels, the degree to which leptin contributed to the differences in feeding behavior and peptide expression is uncertain.

Differences in the circulating metabolic products generated from fat metabolism may have altered hypothalamic peptide expression and feeding behavior. In diabetic rats, ketone bodies are elevated, and in our experiment ketone body levels were significantly higher in the diabetic rats on the HF diet than in any of the other groups. Moreover, ketone body uptake is reported to be higher in the ARC and PVN relative to other regions of the hypothalamus (29). When administered into the CNS of rats, ketones produce a reduction in food intake and body weight (32). Therefore, it is possible that our results are attributable to increased CNS ketone body utilization. On the other hand, this effect would be a complex one because diabetic rats fed the HC diet had elevated ketone body levels relative to nondiabetic rats, yet exhibited the greatest changes in hypothalamic peptide expression.

It should be stressed that the reduction of food intake exhibited by the diabetic rats fed the HF diet is probably not due to an aversive consequence of the HF diet. First, when fed a macronutrient self-selection diet, moderately diabetic rats exhibit a preference for fat over carbohydrate, and the diet ultimately selected by diabetic rats on such a regimen is similar to the HF diets reported by many investigators to be effective in reducing calorie intake (42, 43). Second, diabetic rats prefer flavors paired with the intakes of fat (44). If fat produced aversive effects, animals would be expected to avoid flavors that were paired with those aversive effects. Third, diabetic rats avidly consume corn oil over repeated trials even though oil consumption reduces subsequent food intake (10). Finally, condition taste aversion acquisition in diabetic rats does not occur in response to a HF diet if the diet is not given immediately after the onset of diabetes (45). Taken together, these data suggest that an aversion to the HF diet is unlikely to explain the reduction of food intake in diabetic rats.

In conclusion, the effect of a HF diet in diabetic animals was to reduce food intake to control levels, and attenuate the diabetes-induced changes in hypothalamic NPY and CRH mRNA expression. Moreover, the lack of effect of HF feeding on caloric intake in nondiabetic animals was accompanied by a lack of effect on hypothalamic neuropeptide expression. These observations are consistent with a role for NPY and CRH in the HF-feeding response to diabetes.

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References

1. Leedom, L.J., and W.P. Meehan. 1989. The psychoneuroendocrinology of diabetes mellitus in rodents. *Psychoneuroendocrinology.* 14:275–294.

2. Mayer, J. 1955. Regulation of energy intake and the body weight: the glucostatic and lipostatic hypothesis. *Ann. NY Acad. Sci.* 63:14–42.

3. Panskeep, J., and M. Ritter. 1975. Mathematical analysis of energy regulatory patterns of normal and diabetic rats. *J. Comp. Physiol. Psychol.* 89:1019–1028.

4. Williams, G., J.S. Gill, Y.C. Lee, H.M. Cardoso, B.E. Okpere, and S.R. Bloom. 1989. Increased neuropeptide Y concentrations in specific hypothalamic regions of streptozotocin-induced diabetic rats. *Diabetes.* 38:321–327.

5. White, J.D., D. Olchovsky, M. Kershaw, and M. Berelowitz. 1990. Increased hypothalamic content of preproneuropeptide-Y messenger ribonucleic acid in streptozotocin-diabetic rats. *Endocrinology.* 126:765–772.

6. Sahu, A., C.A. Sninsky, C.P. Phelps, M.G. Dube, P.S. Kalra, and S.P. Kalra. 1992. Neuropeptide Y release from the paraventricular nucleus increases in association with hyperphagia in streptozotocin-induced diabetic rats. *Endocrinology.* 131:2979–2985.

7. Sipols, A.J., D.G. Baskin, and M.W. Schwartz. 1995. Effect of intracerebroventricular insulin infusion on diabetic hyperphagia and hypothalamic neuropeptide gene expression. *Diabetes.* 44:147–151.

8. Marks, J.L., K. Waite, and M. Li. 1993. Effects of streptozotocin-induced diabetes mellitus and insulin treatment on neuropeptide Y mRNA in the rat hypothalamus. *Diabetologia.* 36:497–502.

9. Friedman, M.I. 1978. Hyperphagia in rats with experimental diabetes mellitus: a response to decreased supply of utilizable fuels. *J. Comp. Physiol. Psychol.* 92:109–117.

10. Ramirez, I., and M.I. Friedman. 1983. Food intake and blood fuels after oil consumption: differential effects in normal and diabetic rats. *Physiol. Behav.* 31:847–850.

11. Friedman, M.I., N.K. Edens, I. Ramirez, and J. Granneman. 1983. Food intake in diabetic rats: isolation of primary metabolic effects of fat feeding. *Am. J. Physiol.* 249:R44–R51.

12. Sahu, A., C.A. Sninsky, P.S. Kalra, and S.P. Kalra. 1990. Neuropeptide Y concentration in microdissected hypothalamic regions and in vitro release from the medial basal hypothalamus-preoptic area of streptozotocin-diabetic rats with and without insulin substitution therapy. *Endocrinology.* 126:192–198.

13. Stanley, B.G., and S.F. Leibowitz. 1984. Neuropeptide Y injected into the paraventricular hypothalamus: a powerful stimulant of feeding behavior. *Proc. Natl. Acad. Sci. USA.* 82:3940–3943.

14. Stanley, B.G., S.E. Kyrkouli, S. Lampert, and S.F. Leibowitz. 1986. Neuropeptide Y chronically injected into the hypothalamus: a powerful neurochemical inducer of hyperphagia and obesity. *Peptides.* 7:1189–1192.

15. Krahn, D.D., and B.A. Gosnell. 1988. Behavioral effects of corticotropin-releasing factor: localization and characterization of central effects. *Brain Res.* 443:63–69.

16. Arase, K., N.S. Shargill, and G.A. Bray. 1989. Effect of corticotropin releasing factor on genetically obese (fatty) rats. *Physiol. Behav.* 45:565–570.

17. Glowa, J.R., and P.W. Gold. 1991. Corticotropin releasing hormone produces profound anorexigenic effects in the rhesus monkey. *Neuropeptides.* 18:55–61.

18. Heinrichs, S.C., F. Menzaghi, E. Merlo-Pich, R.L. Hauger, and G.F. Koob. 1993. Corticotropin releasing factor in the paraventricular nucleus modulates feeding induced by neuropeptide Y. *Brain Res.* 611:18–24.

19. Schwartz, M.W., A.J. Sipols, J.L. Marks, G. Sanacora, J.D. White, A. Scheurinck, S.E. Kahn, D.G. Baskin, S.C. Woods, D.P. Figlewicz, and D. Porte, Jr. 1992. Inhibition of hypothalamic neuropeptide Y gene expression by insulin. *Endocrinology.* 130:3608–3616.

20. Dallman, M.F., A.M. Strack, S.F. Akana, M.J. Bradbury, E.F. Hanson,

K.A. Scribner, and M. Smith. 1993. Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. *Front Neuroendocrinol.* 14:303–347.

21. Velasco, A., I. Huerta, and B. Marin. 1988. Plasma corticosterone motor activity and metabolic circadian patterns in streptozotocin-induced diabetic rats. *Chronbiol. Int.* 5:127–135.

22. Strack, A.M., R.J. Sebastian, M.W. Schwartz, and M.F. Dallman. 1995. Glucocorticoids and insulin: reciprocal signals for energy balance. *Am. J. Physiol.* 268:142–149.

23. Ramirez, I., and M.I. Friedman. 1990. Dietary hyperphagia in rats: role of fat, carbohydrate, and energy content. *Physiol. Behav.* 47:1157–1163.

24. Akana, S.F., A.M. Strack, E.S. Hanson, and M.F. Dallman. 1994. Regulation of activity in the hypothalamo-pituitary-adrenal axis is integral to a larger hypothalamic system that determines caloric flow. *Endocrinology.* 135:1125–1134.

25. Wieland, O. 1957. Eine enzymaatische Method zurf Bestimmung von Glycerin. *Biochem. Z.* 329:313–319.

26. Bates, M.W. 1971. Kinetics of ketone body metabolism in fasted and diabetic rats. *Am. J. Physiol.* 221:984–991.

27. Havel, P.J., S. KasimKarakas, W. Mueller, P.R. Johnson, R.L. Gingerich, and J.S. Stern. 1996. Relationship of plasma leptin to plasma insulin and adiposity in normal weight and overweight women: effects of dietary fat content and sustained weight loss. *J. Clin. Endocrinol. Metab.* 81:4406–4413.

28. Landt, M., R.L. Gingerich, P.J. Havel, W.M. Mueller, B. Schoner, J.E. Hale, and M.L. Heiman. 1998. Radioimmunoassay of rat leptin: sexual dimorphism reversed from humans. *Clin. Chem.* 44:565–570.

29. Hawkins, R.A., and J.F. Biebuyck. 1979. Ketone bodies are selectively used by individual brain regions. *Science.* 205:325–327.

30. Giraudo, S.O., C.M. Kotz, M.K. Grace, A.S. Levine, and C.J. Billington. 1994. Rat hypothalamic NPY mRNA and brown fat uncoupling protein mRNA after high-carbohydrate or high-fat diets. *Am. J. Physiol.* 266:R1578–R1583.

31. Havel, P.J., J.Y. Uriu-Hare, T. Liu, K.L. Stanhope, J.S. Stern, C.L. Keen, and B. Ahren. 1998. Marked and rapid decreases of circulating leptin in streptozotocin diabetic rats: reversal by insulin administration. *Am. J. Physiol.* In press.

32. Davis, D.D., D. Wirtshafter, K.E. Asin, and D. Brief. 1981. Sustained intracerebroventricular infusion of brain fuels reduces body weight and food intake in rats. *Science.* 212:81–83.

33. Langhans, W., and E. Scharrer. 1992. Metabolic control of eating, energy expenditure and bioenergetics of obesity. *In* World Review of Nutrition and Dietetics. A.P. Simopoulos, editor. Karger, Basel, Switzerland. 1–67.

34. Zhang, Y., R. Proenca, M. Maffie, M. Barone, L. Leopold, and J.M. Friedman. 1994. Positional cloning of the mouse obese gene and its human homologue. *Nature.* 372:425–432.

35. Campfield, L.A., F.J. Smith, Y. Gulsez, R. Devos, and P. Burn. 1995. Mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science.* 269:546–549.

36. Halaas, J.L., K.S. Gajiwala, M. Maffel, S.L. Cohen, B.T. Chait, D. Rabinowitz, R.L. Lallone, S.K. Burley, and J.M. Friedman. 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science.* 269:543–546.

37. Pelleymounter, M.A., M.J. Cullen, M.B. Baker, R. Hecht, D. Winters, T. Boone, and F. Collins. 1995. Effects of the obese gene product on body weight regulation in *ob/ob* mice. *Science.* 269:540–543.

38. Schwartz, M.W., D.G. Baskin, T.R. Bukowski, J.L. Kuijper, D. Foster, G. Lasser, D.E. Prunkard, D. Porte, S.C. Woods, R.J. Seeley, and D.S. Weigle. 1996. Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in *ob/ob* mice. *Diabetes.* 45:531–535.

39. Weigle, D.S., T.R. Bukowski, D.C. Foster, S. Holderman, J.M. Kramer, G. Lasser, C.E. Lofton-Day, D.E. Prunkard, C. Raymond, and J.L. Kuijper. 1995. Recombinant *ob* protein reduces feeding and body weight in the *ob/ob* mouse. *J. Clin. Invest.* 96:2065–2070.

40. Schwartz, M.W., R.J. Seeley, L.A. Campfield, P. Burn, and D.G. Baskin. 1996. Identification of hypothalamic targets of leptin action. *J. Clin. Invest.* 98:1101–1106.

41. Seeley, R.J., G. van Dijk, L.A. Campfield, F.J. Smith, J.A. Nelligan, S.M. Bell, D.G. Baskin, S.C. Woods, and M.W. Schwartz. 1996. The effect of intraventricular administration of leptin on food intake and body weight in the rat. *Horm. Metab. Res.* 28:664–668.

42. Kanerak, R.B., and L. Ho. 1984. Patterns of nutrient selection in rats with streptozotocin-induced diabetes. *Physiol. Behav.* 32:639–645.

43. Bartness, T.J., and N.E. Rowland. 1983. Diet selection and metabolic fuels in three models of diabetes mellitus. *Physiol. Behav.* 31:539–545.

44. Tordoff, M.G., B.J. Tepper, and M.I. Friedman. 1987. Food flavor preferences produced by drinking glucose and oil in normal and diabetic rats: evidence for conditioning based on fuel oxidation. *Physiol. Behav.* 41:481–487.

45. Baxter, L.C., and P.J. Schofield. 1980. The effects of a high-fat diet on chronic streptozocin-diabetic rats. *Diabetologia.* 18:239–245.