Low-carbohydrate High-fat Diets: Regulation of Energy Balance and Body Weight Regain in Rats

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The aim of the current investigations was to examine the effects of a low-carbohydrate high-fat diet (LC-HFD) on body weight, body composition, growth hormone (GH), IGF-I, and body weight regain after stopping the dietary intervention and returning the diet back to standard laboratory chow (CH). In study one, both adolescent and mature male Wistar rats were maintained on either an isocaloric LC-HFD or CH for 16 days before having their diet switched. In study two, mature rats were maintained on either LC-HFD or CH for 16 days to determine the effects of the LC-HFD on fat pad weight. LC-HFD leads to body weight loss in mature rats ($P < 0.01$) and lack of body weight gain in adolescent rats ($P < 0.01$). Despite less body weight, increased body fat was observed in rats maintained on LC-HFD ($P < 0.05$). Leptin concentrations were higher ($P < 0.05$), and IGF-I ($P < 0.01$) concentrations were reduced in the LC-HFD rats. When the diet was returned to CH following LC-HFD, body weight regain was above and beyond that which was lost ($P < 0.01$). The LC-HFD resulted in increased body fat and had a negative effect upon both GH and IGF-I concentrations, which might have implications for the accretion and maintenance of lean body mass (LBM), normal growth rate and overall metabolic health. Moreover, when the LC-HFD ceases and a high-carbohydrate diet follows, more body weight is regained as compared to when the LC-HFD is consumed, in the absence of increased energy intake.

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INTRODUCTION

Low-carbohydrate high-fat diets (LC-HFDs), such as the Atkins diet (1), are most commonly known for their weight reducing properties. A number of investigations have reported beneficial effects of LC-HFDs for weight loss (2–6), yet the mechanisms underlying the effectiveness of such diets are unknown. Proposed, but unconfirmed, contributing factors include altered appetite regulation (7–9) reduced availability of foods (2) or loss of total body water, especially in the initial phase (10,11). Proponents of LC-HFDs (1) suggest an increase in basal energy expenditure (EE) occurs in individuals following the diet thus contributing to weight loss. This was investigated in moderately obese females over a period of 4 months (3), but no effects of a LC-HFD were observed on EE. When one proportion of the diet is reduced then naturally another proportion increases, in this case the reduction in carbohydrate (CHO) is replaced by dietary fat. Many concerns regarding the extremely high-fat content of such diets have been expressed over the past few years (8); however, the diet still remains a popular choice for many individuals who seek to lose weight.

LC-HFDs have been reported to be very well tolerated, especially in comparison to low-fat diets used for weight loss (12). Interestingly though, several pieces of evidence suggest that consuming a LC-HFD is intolerable for some individuals (4,13). In the case that individuals find the diet hard to adhere to because of the extreme CHO restriction, for example, other methods of weight loss may be adopted such as the transition from a LC-HFD to a low-fat diet. In a randomized crossover design investigation, Volek *et al.* (14) reported that 20% of males and 30% of females actually regained more body weight than they had lost when the diet was changed from a very low-CHO diet to a low-fat diet despite no difference in energy intake. Whether isocaloric diets differing in macronutrient composition have differential effects on body weight loss, body composition and body weight regain once the diet ceases to continue remains to be further investigated.

What is less known is that LC-HFDs are also useful for the treatment of intractable epilepsy in children (15–18). One concern regarding the use of such diets for the treatment of epilepsy is that the diet has been demonstrated to affect the normal longitudinal growth especially in younger children (19–21).

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One contributing factor to the lack of normal growth may be the effect of the diet on body composition and possibly insufficient lean body mass (LBM) accretion. For example, previous reports demonstrate that the consumption of a diet high in fat results in an elevated plasma somatostatin, a potent inhibitor of growth hormone (GH) (22,23), additionally elevation of free fatty acids and insulin concentrations might also mediate this effect (24). Patients deficient in GH demonstrate altered body composition, usually presenting as increased fat mass (particularly visceral fat) because of decreased lipolysis and decreased LBM. Reduced exercise capacity and muscle strength has also been reported in these patients (24). Increased body fat, despite no differences in bodyweight, has been reported in rodents maintained on a LC-HFD (9,25,26). Therefore, consuming a LC-HFD either for weight loss or for the treatment of epilepsy can affect upon body composition. One such mechanism may be the suppression of GH secretion resulting in reduced IGF-I levels due to elevated expression of somatostatin.

In the current investigation, we examine the effects of short-term exposure to a LC-HFD on body weight, body fat, and on physiological as well as surrogate plasma parameters of energy balance. The LC-HFD used in the current investigations is lower in total protein, although adequate for normal growth, and lower in CHO content as to what is usually recommended for body weight loss (Atkins style diet) and the for the treatment of epilepsy. Although relatively extreme in composition, the current LC-HFD was chosen to examine the effects of severe CHO restriction while at the same time limiting gluconeogenesis from dietary protein. Additionally we examine the effects of the LC-HFD on body weight regain when the diet is return to standard laboratory chow (CH). Because of the known effect of the LC-HFD on growth rates in some children and adolescents the current investigation was conducted on adolescent and mature rats to further explore differential responses to a LC-HFD.

METHODS AND PROCEDURES

Animals

In study one, 32 male Wistar rats (Harlan-Winkelmann, Germany; ~10 weeks old; 301 ± 3.4 g (mean ± s.d.) $n = 16$; and, ~16 weeks old 429 ± 3.2 g; $n = 15$) were included. In study two, 16 male Wistar rats (Harlan-Winkelmann, Germany, ~17 weeks old; 446 ± 5.6 g) were also used. For the purposes of the article, the animals will be referred to as adolescent (10 weeks) and mature (16–17 weeks). Animals were housed in individual cages (21.8 ± 0.3 °C: humidity 70 ± 1.0%) and maintained on a 12-h light–dark cycle throughout the study (lights on at 0200 h and off at 1400 h). All animals received ad libitum access to standard laboratory CH for the first 10 days following delivery to allow acclimation to the new environment. Body weight and 24-h food intake was measured daily (Sartorius Competence CP2201) 1 h before the onset of the dark period. At the end of the acclimation period, animals were divided into two weight-matched groups. All procedures were approved by Upper Bavarian Government's ethical committee for animal experiments (AZ 55.2-1-54-2531-47-05).

Study protocol and diets

A crossover design was used in study one for this investigation as all animals were exposed to both standard laboratory CH (% of energy: 9% fat, 33% protein, 58% CHO, 3.04 kcal/g) and the LC-HFD (94% fat, 4.2% protein and 1.3% CHO, 7.5 kcal/g, Sniff, Soest, Germany) for either the first 16 days or the latter 16 days of the experiment. Animals maintained on the LC-HFD first were pair-fed (kcals) with weight-matched controls exposed to ad libitum CH first. In the second phase of the experiment pair-feeding was switched over between groups. In each phase, all animals were pair-fed with ad libitum CH-fed controls in the same age groups. This ensured that any effects observed were due to the macronutrient composition of the diet and not due to difference in caloric intake between groups and experimenters verified daily that all LC-HFD fed animals consumed all the food allocated to them. Measurements of EE and respiratory quotient (RQ) were made at baseline, 16 days following exposure to the first diet, and 16 days following exposure to the second diet (study one). Metabolic assessments (EE, RQ) were made via indirect calorimetry (CalsoSys, TSE Systems, Bad Homburg, Germany). In study two, mature rats were maintained on either CH or the LC-HFD for 16 days. All animals were given ad libitum access to water throughout the experimental period. At the end of both of the experiments all animals were given access to food for 1 h after lights out, they were then fasted for 6 h and killed under isofluran anesthesia. Trunk blood was collected for hormone analysis and samples were then stored at −80 °C degrees until analysis. In study two, fat pads were also collected and weighed immediately.

Assays

All hormones were measured with commercially available kits as per manufacturers instructions, (serum) GH (ACTIVE Mouse/Rat Growth Hormone; DSL, Webster, TX), insulin (Sensitive Rat Insulin Ria Kit, Linco, St Charles, Missouri), leptin (ACTIVE Murine Leptin ELISA; DSL), IGF-I (Rat/Mouse IGF-I; IDS, Tyne & Wear, UK), glucose (glucose oxidase method, EcoSolo, Care Diagnostica, Voerde, Germany), albumin (Cobas Integra 800; Roche Diagnostics, Mannheim, Germany).

Data analysis

Data are presented as means ± s.e.m. and statistical analyses were performed using SPSS for Windows (v14.0; SPSS, Chicago, IL). Repeated-measures ANOVA was used to asses changes in body weight on each diet (CH, LC-HFD), with age (adolescent, mature) and diet order (CH first vs. LC-HFD first) as factors. Independent groups t-test was used to further explore a significant three-way interaction. Ad libitum CH intake was assessed with ANOVA, with age and diet order as factors. As we were interested in body weight regain following maintenance on a LC-HFD one-way ANOVA was performed to compare feeding efficiency between adolescent and mature rats that consumed CH in the first phase and those who consumed CH in the second phase. Bonferroni was used to indicate where significant differences exist between groups. Feeding efficiency was calculated over the 16 days ad libitum CH feeding period for all groups; this was the ratio of the number of calories ingested to the amount of weight gain (kcal/g $\times 10^3$) (27). Repeated measures $2 \times 2 \times 3$ ANOVA with age, diet order and day (baseline, post 16 days of diet 1 and post 16 of diet 2) as factors was used to examine EE and RQ. One-way ANOVAs were performed on each group where a three-way interaction existed, with pairwise comparisons (LSD, equivalent to no adjustments) used to indicate significant differences between days. Univariate analysis of variance was used to examine significant differences in hormones, with age and terminal diets (CH, LC-HFD) as factors. In study two, t-tests were used to examine differences in body weight change and fat pad weight. The alpha value chosen was 0.05.

RESULTS

Bodyweight change

Body weight change (**Figure 1**) was examined to investigate the effects of maintaining rodents on a LC-HFD in comparison with maintenance on CH. Overall, irrespective of age and the order in which the diets were consumed, significantly more body weight was lost when the animals were maintained on LC-HFD in comparison to CH. The average change in body weight of the

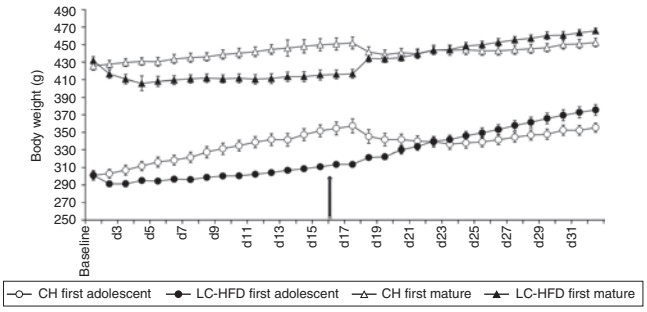

Figure 1 Body weight development over the whole experimental period, of adolescent and mature rats maintained on either chow first or a low-carbohydrate high-fat diet (LC-HFD) first for 16 days before having their diets switched (arrow indicates the diet switch) for a further 16 days (means ± s.e.m.).

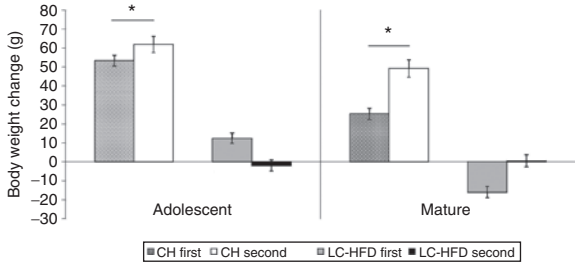

Figure 2 Body weight change in adolescent and mature rats (g, means ± s.e.m.) following 16 days maintenance on chow (CH) as the first diet and body weight change in animals maintained on CH as the second diet, following 16 days maintenance on the LC-HFD. (*$P < 0.01$).

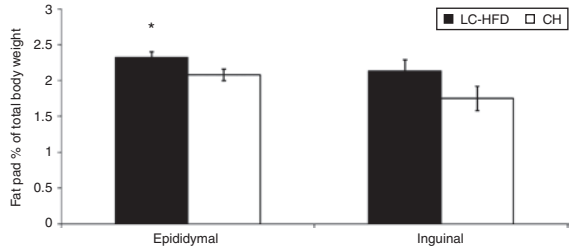

Figure 3 Epididymal and inguinal fat pads (% of total body weight) in old rats maintained on chow (CH) or low-carbohydrate high-fat diet (LC-HFD) for 16 days.

CH-fed animals was 113% of the LC-HFD-fed animals. (CH 47 ± 2 g vs. LC-HFD −1 ± 2 g; $P < 0.01$, **Figure 2**). Consuming CH following 16 days maintenance on the LC-HFD resulted in increased body weight gain. Overall animals that consumed CH in the second study phase following 16 days exposure to LC-HFD demonstrated increased body weight gain in comparison to animals maintained on CH, the mean body weight change of the LC-HFD animals was 72% of the CH-fed animals (CH 2nd 27 ± 1 g vs. CH 1st 19 ± 1 g; $P < 0.01$).

Maintenance on either standard laboratory CH or the LC-HFD resulted in significantly different body weight change depending on the age of the animal (adolescent vs. mature) and the order in which each diet was consumed (CH first or LC-HFD first). This was indicated by a significant three-way interaction ($P < 0.01$). Mature animals demonstrated body weight loss (−16 ± 3 g) in comparison to adolescent animals who demonstrated a lack of body weight gain (12 ± 3 g; $P < 0.01$). Mature rats maintained on CH in the first phase gained 163% the body weight of the LC-HFD-fed mature rats in the first phase, (25 ± 3 g vs. −16 ± 3 g) ($P < 0.01$), demonstrating body weight loss in this group of animals. Body weight gain between the adolescent rodents was significantly different in the first phase, with the LC-HFD gaining 23.29% of that of the CH-fed rats; adolescent animals maintained on CH in the first phase gained more weight in comparison to the adolescent LC-HFD fed animals (53 ± 3 g vs. 12 ± 3 g) ($P < 0.05$). On average adolescent rodents maintained on LC-HFD gained 0.8 g per day of body weight in comparison to 3.3 g per day in the CH-fed controls. In response to the diet switch, significant differences in body weight gain were only found in mature animals who were maintained on CH in the second phase in comparison to those maintained on CH in the first phase, mean body weight gain in the first phase was only 51% of that gained in the second phase of CH feeding (CH first 25 ± 3 g vs. CH second 49 ± 3 g; $P < 0.01$). Although not significant, the adolescent animals gained slightly more body weight, when CH was consumed in the second phase. Average body weight gain in the first CH-feeding phase was 86% of that gained in the second CH-feeding phase (CH first 53 ± 3 g, CH second 62 ± 5 g) ($P > 0.05$).

In study two, mature rats maintained on the LC-HFD gained significantly less body weight in comparison to CH-fed controls (CH 27 ± 1 g vs. LC-HFD 2 ± 3 g; $P < 0.01$), with the LC-HFD-fed animals gaining only 7% of that gained by the CH-fed group. Epididymal (**Figure 3**) fat pad weights as expressed as a percentage of total body weight were significantly higher in the LC-HFD rat as compared to those maintained on CH (2.32 ± 0.08% vs. 2.05 ± 0.08%; $P < 0.05$). Yet, inguinal fat pads did not differ between groups (2.13 ± 0.16% vs. 1.75 ± 0.17%; $P > 0.05$). Absolute fat pad weights (g) did not differ significantly between groups (epididymal: CH 9.7 ± 0.47 g, LC-HFD 10.45 ± 0.37 g; $P > 0.05$, Inguinal: CH 8.36 ± 0.97 g, LC-HFD 9.62 ± 0.71 g; $P > 0.05$) despite less body weight gain in the LC-HFD groups.

Ad libitum CH intake and feeding efficiency

The average CH intake (kcal) was analyzed (**Figure 4a** average intake (kcal), **Figure 4b** daily energy intake (kcal)). Overall, animals that consumed CH as their first diet had significantly elevated food intake (74.40 ± 1.36 kcal) in comparison to those animals who consumed CH second (69.22 ± 1.32 kcal; $P < 0.05$). Feeding efficiency was significantly elevated in the mature animals who consumed CH second (42.55 ± 7.51) in comparison to the animals who were maintained on CH first (21 ± 6.77; $P < 0.01$) and in the adolescent animals who consumed the CH second (57.93 ± 11.20) in comparison to the adolescent animals who consumed CH first (46.01 ± 5.86; $P < 0.05$).

EE and RQ

EE measurements (**Table 1**) were significantly different over the three measurement days, thus suggesting that EE differed according to the dietary manipulation. ANOVA revealed a

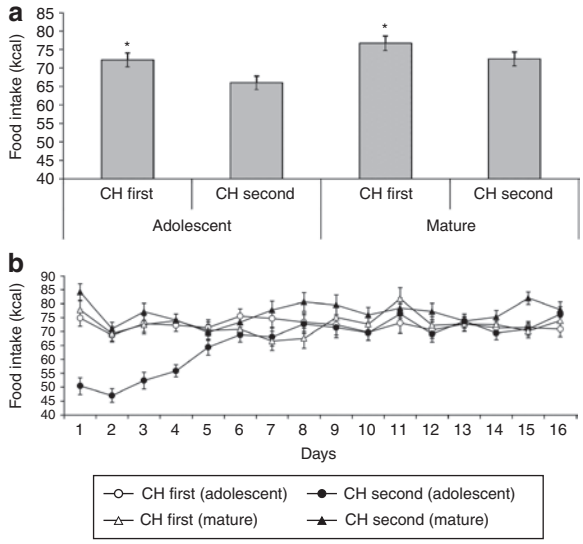

Figure 4 Ad libitum food intake. (**a**) Ad libitum average intake over a 16 day period (kcal, means ± s.e.m.) in animals maintained on chow (CH) as the first diet, and CH as the second diet following 16 days maintenance on the LC-HFD. (ANOVA main effect of diet order, $^*P < 0.01$). (**b**) Daily caloric intake in CH-fed animals used for pair feeding.

Table 1 Energy expenditure (EE = kcal/kg/h) and RQ in adolescent and mature rats, measured for 22 h at baseline and 16 days following maintenance on chow (CH) and a low-carbohydrate high-fat diet (LC-HFD)

		Baseline	Post 16 days of diet 1	Post 16 days diet 2
CH 1st adolescent	EE	7.74 (0.09)	7.14 (0.08)[a]	6.25 (0.2)[a,b]
	RQ	1.07 (0.12)	1.02 (0.01)[a]	0.81 (0.1)[a]
CH 1st mature	EE	6.13 (0.10)	5.91 (0.10)	5.435 (0.16)[a,b]
	RQ	1.10 (0.01)	1.03 (0.01)[a]	0.81 (0.01)[a]
LC-HFD 1st adolescent	EE	7.43 (0.05)	6.96 (0.21)	7.27 (0.16)
	RQ	1.13 (0.02)	0.76 (0.01)[a]	1.06 (0.01)[a,b]
LC-HFD 1st mature	EE	6.48 (0.07)	5.69 (0.08)[a]	5.97 (0.11)[a]
	RQ	1.07 (0.02)	0.78 (0.01)[a]	1.07 (0.01)[b]

One-way ANOVA.
[a]Statistically significant difference from baseline. [b]Statistically significant from diet 1, means ± s.e.m.

significant three-way interaction ($P < 0.01$). In the mature animals who were given the LC-HFD first, a significant reduction in EE was observed in comparison to baseline ($P < 0.01$), yet switching the diet back to CH after 16 days of consuming the LC-HFD did not result in an increase in EE in comparison to baseline ($P > 0.05$). Although not significant, adolescent animals also demonstrated a tendency for reduced EE following exposure to the LC-HFD. When EE was normalized to body mass and expressed for a period of 24 h (Table 2) maintenance on the LC-HFD resulted in a reduction ($P < 0.01$) in EE in all groups except for the adolescent animals maintained on the LC-HFD first where a reduction in EE was observed; however, the decline was again nonsignificant. Additionally, when normalized for body mass, returning the diet to regular

Table 2 Energy expenditure (kcal/24 h) normalized for body mass at baseline and 16 days following maintenance on chow (CH) and a low-carbohydrate high-fat diet (LC-HFD)

	Baseline	Post 16 days of diet 1	Post 16 days of diet 2
CH 1st adolescent	55.88 (1.08)	60.70 (1.41)[a]	53.29 (1.60)[b]
CH 1st mature	62.55 (1.02)	63.85 (1.36)	58.86 (1.23)[a,b]
LC-HFD 1st adolescent	53.72 (0.86)	52.39 (1.61)	65.39 (1.22)[a,b]
LC-HFD 1st mature	67.18 (0.91)	56.75 (0.77)[a]	66.53 (1.11)[b]

One-way ANOVA.
[a]Statistically significant difference from baseline. [b]Statistically significant from diet 1, means ± s.e.m.

CH after maintenance on the LC-HFD first resulted in an increase in EE comparable to baseline in both adolescent and mature rats.

Similar to EE, RQ varied according to the diet manipulation (Table 1). ANOVA revealed a significant three-way interaction for RQ ($P < 0.01$). Consuming the LC-HFD resulted in a consistent reduction to RQ in all animals ($P < 0.01$). When the animals that had been maintained on LC-HFD for the first 16 days had their diet returned back to CH, RQ significantly increased ($P < 0.01$). In mature animals (LC-HFD 1st) RQ, following 16 days exposure to CH, RQ returned to baseline levels ($P < 0.01$) but this was not the case in the adolescent animals, RQ remained significantly lower in comparison to baseline values ($P < 0.01$).

Effects on endocrine system

In study one (adolescent and mature rats) ANOVA revealed a main effect of diet for IGF-I, leptin, insulin, glucose, and plasma albumin. IGF-I was significantly decreased in the LC-HFD group (1,693.4 ± 58.3 vs. 922 ± 60.9 ng/ml; $P < 0.01$), Growth hormone was also decreased, although not significantly, in the LC-HFD group (53.51 ± 11.84 vs. 19.62 ± 12.26 ng/ml; $P = 0.057$). LC-HFD animals had significantly elevated leptin levels (3.44 ± 0.28 vs. 1.27 ± 0.35 ng/ml; $P < 0.05$). Insulin and glucose concentrations were significantly lower in the LC-HFD animals (0.27 ± 0.06 vs. 0.60 ± 0.05 ng/ml; $P < 0.01$) and (5.66 ± 0.36 vs. 7.93 ± 0.35 mmol/l; $P < 0.01$). ANOVA revealed no significant main effect of age or age × diet interaction for IGF-I, GH, leptin, insulin, or glucose. Overall plasma albumin was significantly lower in CH-fed animals (3.48 ± 0.051 vs. 3.63 ± 0.52 g/dl; $P < 0.05$), ANOVA revealed a significant age × diet interaction, with no differences occurring between adolescent rats ($P > 0.05$), yet mature rats displayed significant differences ($P < 0.05$). (Table 3 shows data for adolescent and mature animals separately).

Study two (mature rats only) confirmed data obtained from study one in the mature rats, IGF-I was significantly lower in the LC-HFD fed animals (1,076.3 ± 116.1 vs. 1,456.12 ± 166.6 ng/ml; $P < 0.001$). Leptin concentrations were significantly higher in the LC-HFD fed animals (2.08 ± 0.23 vs. 1.12 ± 0.21 ng/ml; $P < 0.05$) and plasma albumin was

Table 3 End-point hormone, glucose, and albumin analysis (study 1, mean ± s.e.m.) in adolescent and mature rats maintained for the last 16 days (second diet phase) on either chow (CH) or a low-carbohydrate high-fat diet (LC-HFD)

	Adolescent CH	Adolescent LC-HFD	Mature CH	Mature LC-HFD	Main effect of diet	Main effect of age
IGF-I (ng/ml)	1757.3 ± 85.2	893.9 ± 79.70	1629.5 ± 79.7	951.8 ± 92.0	$P < 0.01$	$P > 0.05$
GH (ng/ml)	50.1 ± 16.7	12.9 ± 16.5	56.9 ± 16.7	26.3 ± 17.9	$P = 0.057$	$P > 0.05$
Leptin (ng/ml)	1.25 ± 0.5	2.47 ± 0.4	1.29 ± 0.4	4.4 ± 0.4	$P < 0.05$	$P = 0.052$
Insulin (ng/ml)	0.72 ± 0.11	0.32 ± 0.10	0.60 ± 0.5	0.21 ± 0.04	$P < 0.01$	$P > 0.05$
Glucose (mmol/l)	8.2 ± 0.5	5.5 ± 0.5	7.65 ± 0.5	5.8 ± 0.5	$P < 0.01$	$P > 0.05$
Albumin (g/dl)	3.54 ± 0.04	3.55 ± 0.11	3.42 ± 0.07	3.72 ± 0.07	$P < 0.05$	$P < 0.05$

significantly lower in CH-fed animals (3.92 ± 0.05 vs. 4.16 ± 0.18 g/dl; $P < 0.05$).

DISCUSSION

In the current investigations, we were able to both confirm and extend existing knowledge regarding the effects of LC-HFDs on body weight (2–7) in addition to highlighting the effects on the energy balance system once the diet is terminated and the habitual diet is resumed. A lack of body weight gain was observed in adolescent animals on a LC-HFD and a reduction in body weight, in heavier, mature animals maintained on a LC-HFD under isocaloric conditions. Few investigations have been carried out examining the effect of reverting back to the habitual diet following maintenance on a LC-HFD on body weight regain. When the LC-HFD was replaced by ad libitum CH, body weight gain exceeded that of those animals maintained on standard CH for the first 16 days. In the mature animals, body weight regain was above and beyond that what was lost during maintenance on a LC-HFD. In addition, it appears that EE (both absolute and body mass standardized) in this instance was reduced as a result of consuming a LC-HFD for 16 days in the majority of animals. Consuming a LC-HFD may alter body composition since increased fat mass and leptin concentrations were observed, and hormones known to increase LBM (GH, IGF-I) were decreased. At face value, it appears that a LC-HFD may be an effective tool for weight loss; however, the consumption of a LC-HFD has several implications for body composition, growth rate, and also for body weight regain when the LC-HFD ceases to be consumed.

Rodents that have different initial body weights demonstrate differential responses to the consumption of a LC-HFD, thus highlighting the importance of age or the starting body weight in such investigations. The observed effect in adolescent animals was a lack of body weight gain (28–30) and the mature, heavier animals demonstrated body weight loss (2,3,5,7,12). In contrast, other investigations examining the effect of a LC-HFD on body weight in rodents have, however, failed to demonstrate any changes to body weight in comparison to the CH controls (9,25,26). Discrepancies in findings may be due to the strain of rat used, plus the older rats in this investigation were heavier at around 429 g as compared with the range of 215–270 g in the investigations where no effects were observed.

In the current investigation, increased fat mass was found in the LC-HFD fed rats. We also speculate that these animals had a lower percentage of LBM since endocrine parameters related to body composition (IGF-I and GH), in particular LBM, were reduced in this group of rats. Hormone analysis revealed that animals consuming the LC-HFD for 16 days resulted in decreased GH and IGF-I. Interestingly, two human studies demonstrated that that IGF-I concentrations are positively correlated with physical fitness levels (31,32). A decline in IGF-I and reduced LBM, in addition to reduced glycogen availability (10), may contribute to the mechanisms underlying increased perceived exhaustion in individuals consuming low-CHO diets (33–35). Limited CHO intake also results in reduced insulin concentrations and this has been linked to increased urinary nitrogen excretion (36) although this parameter was not measured in the current investigation. Insulin is a potent anabolic hormone, primarily inhibiting proteolysis (37,38) and so the extremely low-CHO content of the LC-HFD may in part contribute to the negative effect of a LC-HFD on LBM. In the current investigation higher leptin levels were found in the animals maintained on the LC-HFD (9,29). Increased leptin is a good indicator of BMI (39) and increased fat mass (40,41) which is consistent with the findings of increased body fat (9,25,26). Furthermore, if alterations in body composition do occur with our diet, in particular a decline in LBM reflective of decreased muscle mass, it may partially explain the reduction in EE in the current investigation (42). We observed a decline in both absolute and normalized (for body mass) EE in all groups of animals maintained on the LC-HFD in the first and second phases, with the exception of the adolescent rats that were given the LC-HFD as the first diet, where a rather modest decline was observed. However, it could be the case that the changes observed in the more mature rats are more relevant to the human adult population following similar diets, due to the mature rat having a more stable energy homeostasis system in comparison to adolescent rats. The reduction in EE in the LC-HFD group could also be a consequence of reduced physical activity levels (43–45). Exercise may play a role in the preservation of LBM in individuals consuming a LC-HFD (6), yet this assertion warrants further investigation.

One important consideration of the current investigation is the low protein content of the diet used, adequate protein in the diet is required to maintain LBM. While the diet composition used in this investigation is not reflective of "real-life"

low-CHO diets typically used in humans (Atkin's diet), because they are higher in protein, results of the current investigation provide useful insight to the effects of limiting CHO intake. Additionally, the National Research council (NRC 1995; Nutrient Requirements of Laboratory Animals) (46) protein requirement is 5% CP for body mass maintenance for rats and 15% for growing rats. Although the adolescent rats in the current investigation were still growing, although very slowly, the protein content and amino acid content of the LC-HFD is adequate and is unlikely to cause amino acid deficiency/negative protein balance since the LC-HFD fed animals did not have reduced albumin concentrations. In previous investigations demonstrating increased percentage of body fat (9,26) in animals maintained on a low-CHO diet, one interesting aspect is that the diets that were used were higher in protein content (15% and 35% of energy). Therefore, this indicates that the possible effects of a LC-HFD on body composition observed in the current investigation are not due to diet induced amino acid deficiency. While the rodents maintained on the LC-HFD in the current investigation were not exposed to ad libitum feeding conditions per se, rather isocaloric feeding conditions, it remains to be further investigated whether the apparent effects of consuming such a diet on body composition persists with ad libitum feeding. This approach would provide further insight as to whether the method of feeding, in addition to the diet composition resulted in LBM degradation or lack of accretion of LBM.

Hypersensitivity to weight gain following exposure to LC-HFD

There is a paucity of data existing that examines the effects of returning back to the habitual diet on body weight regain, following exposure to extreme diets such as an Atkins style diet. Body weight loss achieved by severely limiting CHO intake may not be easily sustainable due to possible alterations in body composition and to the energy balance system. Here we demonstrate in a rodent model that following short-term exposure to a LC-HFD diet results in increased body weight gain once the habitual diet is resumed, despite the relatively low-fat content of the habitual diet (9% fat). Interestingly, this increase in body weight gain also occurred despite the fact that ~7% less energy was consumed in animals fed ad libitum CH in phase two. By the end of 16 days exposure to ad libitum CH, following the LC-HFD, the mature rats had not only regained all the weight that they lost on the LC-HFD but had also gained an additional amount and feeding efficiency was elevated by around 50% in the mature animals and 20% in the adolescent animals maintained on CH following the LC-HFD. The energy cost of weight gain in this instance appears to be altered; metabolic efficiency is enhanced, so calorie for calorie more body weight gain is observed. Similar findings have been reported in response to ad libitum food exposure following caloric restriction (27), demonstrating an increased drive to regain the body weight lost. Over time such body weight loss/gain cycles with LC-HFDs may eventually lead to greater weight gain and endocrine disruptions related to energy balance and body composition.

Another factor contributing to elevated body weight gain could be partly due an increase in CHO oxidation or a decrease in fat oxidation since RQ was elevated when the diet was reverted to CH following the LC-HFD. This shift in fuel utilization promotes the deposition of fat (47,48) thus contributing to increased body mass. We acknowledge and can currently provide no scientific explanation with regards to the decline in the RQ in the animals maintained on CH first. Yet, it seems that the more potent decline was indeed observed when the LC-HFD was consumed, as well as the more relevant drastic increase in RQ when animals were fed CH as the second diet. Insulin levels were also higher in animals consuming CH, another strong contributing factor to increased fat deposition (49). In addition to these endocrine factors, body weight regain might also be due to increased total body water due the increase in CHO content of the diet leading to increased glycogen and water storage, however, this is only likely to occur over the first few days of the diet change.

In summary, here we demonstrate that short-term exposure to an isocaloric LC-HFD results in body weight loss, suggesting that merely altering the macronutrient composition of the diet is sufficient to achieve changes in body weight. Yet, increased body weight gain occurs, above and beyond that what was lost once the diet is stopped and the habitual diet or even a low-fat diet is resumed in the absence of increased energy intake. This effect may be partially explained by an increase in the metabolic efficiency of ingested nutrients following exposure to extreme diets such as an Atkins style diet. Second, dietary interventions such as LC-HFD appear to alter the energy balance system, possibly causing reductions in LBM and therefore EE. Additionally and poignantly, LC-HFD not only appears to alter body composition in adult rats, but may also impair normal growth in adolescent rats, resulting in reduced or lack of accretion of LBM. Therefore, great caution may be required when prescribing the use of such diets for weight loss or intractable epilepsy in children and adolescents.

DISCLOSURE

The authors declared no conflict of interest.

REFERENCES

1. Atkins R. *Dr. Atkins New Diet Revolution*. HarperCollins: New York, 1992.
2. Brehm BJ, Seeley RJ, Daniels SR, D'Alessio DA. A randomized trial comparing a very low carbohydrate diet and a calorie-restricted low fat diet on body weight and cardiovascular risk factors in healthy women. *J Clin Endocrinol Metab* 2003;88:1617–1623.
3. Brehm BJ, Spang SE, Lattin BL *et al*. The role of energy expenditure in the differential weight loss in obese women on low-fat and low-carbohydrate diets. *J Clin Endocrinol Metab* 2005;90:1475–1482.
4. Foster GD, Wyatt HR, Hill JO *et al*. A randomized trial of a low-carbohydrate diet for obesity. *N Engl J Med* 2003;348:2082–2090.
5. Samaha FF, Iqbal N, Seshadri P *et al*. A low-carbohydrate as compared with a low-fat diet in severe obesity. *N Engl J Med* 2003;348:2074–2081.
6. Volek JS, Sharman MJ, Love DM *et al*. Body composition and hormonal responses to a carbohydrate-restricted diet. *Metabolism* 2002;51:864–870.
7. Volek JS, Westman EC. Very-low-carbohydrate weight-loss diets revisited. *Cleve Clin J Med* 2002;69:849, 853, 856–858 passim.
8. Astrup A, Meinert Larsen T, Harper A. Atkins and other low-carbohydrate diets: hoax or an effective tool for weight loss? *Lancet* 2004;364:897–899.

9. Kinzig KP, Scott KA, Hyun J, Bi S, Moran TH. Altered hypothalamic signaling and responses to food deprivation in rats fed a low-carbohydrate diet. *Obes Res* 2005;13:1672–1682.
10. Adam-Perrot A, Clifton P, Brouns F. Low-carbohydrate diets: nutritional and physiological aspects. *Obes Rev* 2006;7:49–58.
11. Bray GA. Low-carbohydrate diets and realities of weight loss. *JAMA* 2003; 289:1853–1855.
12. Yancy WS Jr, Olsen MK, Guyton JR, Bakst RP, Westman EC. A low-carbohydrate, ketogenic diet versus a low-fat diet to treat obesity and hyperlipidemia: a randomized, controlled trial. *Ann Intern Med* 2004;140:769–777.
13. Stern L, Iqbal N, Seshadri P *et al*. The effects of low-carbohydrate versus conventional weight loss diets in severely obese adults: one-year follow-up of a randomized trial. *Ann Intern Med* 2004;140:778–785.
14. Volek J, Sharman M, Gomez A *et al*. Comparison of energy-restricted very low-carbohydrate and low-fat diets on weight loss and body composition in overweight men and women. *Nutr Metab (Lond)* 2004;1:13.
15. Gasior M, Rogawski MA, Hartman AL. Neuroprotective and disease-modifying effects of the ketogenic diet. *Behav Pharmacol* 2006;17:431–439.
16. Hartman AL, Vining EP. Clinical aspects of the ketogenic diet. *Epilepsia* 2007;48:31–42.
17. Kang HC, Lee HS, You SJ, Kang du C, Ko TS, Kim HD. Use of a modified Atkins diet in intractable childhood epilepsy. *Epilepsia* 2007;48:182–186.
18. Sinha SR, Kossoff EH. The ketogenic diet. *Neurologist* 2005;11:161–170.
19. Liu YM, Williams S, Basualdo-Hammond C, Stephens D, Curtis R. A prospective study: growth and nutritional status of children treated with the ketogenic diet. *J Am Diet Assoc* 2003;103:707–712.
20. Vining EP, Pyzik P, McGrogan J *et al*. Growth of children on the ketogenic diet. *Dev Med Child Neurol* 2002;44:796–802.
21. Williams S, Basualdo-Hammond C, Curtis R, Schuller R. Growth retardation in children with epilepsy on the ketogenic diet: a retrospective chart review. *J Am Diet Assoc* 2002;102:405–407.
22. Penman E, Wass JA, Medbak S *et al*. Response of circulating immunoreactive somatostatin to nutritional stimuli in normal subjects. *Gastroenterology* 1981;81:692–699.
23. Cappon JP, Ipp E, Brasel JA, Cooper DM. Acute effects of high fat and high glucose meals on the growth hormone response to exercise. *J Clin Endocrinol Metab* 1993;76:1418–1422.
24. Hoffman AR. Treatment of the adult growth hormone deficiency syndrome: directions for future research. *Growth Horm IGF Res* 2005;15(Suppl A): 48–52.
25. Lapachet RA, Miller WC, Arnall DA. Body fat and exercise endurance in trained rats adapted to a high-fat and/or high-carbohydrate diet. *J Appl Physiol* 1996;80:1173–1179.
26. McNeel RL, Mersmann HJ. Low- and high-carbohydrate diets: body composition differences in rats. *Obes Res* 2005;13:1651–1660.
27. MacLean PS, Higgins JA, Jackman MR *et al*. Peripheral metabolic responses to prolonged weight reduction that promote rapid, efficient regain in obesity-prone rats. *Am J Physiol Regul Integr Comp Physiol* 2006;290:R1577–R1588.
28. Nylen K, Likhodii S, Abdelmalik PA, Clarke J, Burnham WM. A comparison of the ability of a 4:1 ketogenic diet and a 6.3:1 ketogenic diet to elevate seizure thresholds in adult and young rats. *Epilepsia* 2005;46:1198–1204.
29. Thio LL, Erbayat-Altay E, Rensing N, Yamada KA. Leptin contributes to slower weight gain in juvenile rodents on a ketogenic diet. *Pediatr Res* 2006;60:413–417.
30. Zhao Q, Stafstrom CE, Fu DD, Hu Y, Holmes GL. Detrimental effects of the ketogenic diet on cognitive function in rats. *Pediatr Res* 2004;55:498–506.
31. Kelly PJ, Eisman JA, Stuart MC *et al*. Somatomedin-C, physical fitness, and bone density. *J Clin Endocrinol Metab* 1990;70:718–723.
32. Poehlman ET, Copeland KC. Influence of physical activity on insulin-like growth factor-I in healthy younger and older men. *J Clin Endocrinol Metab* 1990;71:1468–1473.
33. Burke LM, Hawley JA, Angus DJ *et al*. Adaptations to short-term high-fat diet persist during exercise despite high carbohydrate availability. *Med Sci Sports Exerc* 2002;34:83–91.
34. Johnston CS, Tjonn SL, Swan PD, White A, Hutchins H, Sears B. Ketogenic low-carbohydrate diets have no metabolic advantage over nonketogenic low-carbohydrate diets. *Am J Clin Nutr* 2006;83:1055–1061.
35. Phinney SD. Ketogenic diets and physical performance. *Nutr Metab (Lond)* 2004;1:2.
36. Bisschop PH, Pereira Arias AM, Ackermans MT *et al*. The effects of carbohydrate variation in isocaloric diets on glycogenolysis and gluconeogenesis in healthy men. *J Clin Endocrinol Metab* 2000;85: 1963–1967.
37. Harber MP, Schenk S, Barkan AL, Horowitz JF. Effects of dietary carbohydrate restriction with high protein intake on protein metabolism and the somatotropic axis. *J Clin Endocrinol Metab* 2005;90:5175–5181.
38. Volek JS, Forsythe CE. Diet, exercise and hormone interactions on skeletal muscle. *Horm Res* 2006;66(Suppl 1):17–21.
39. Ahima RS, Osei SY. Leptin signaling. *Physiol Behav* 2004;81:223–241.
40. Considine RV, Sinha MK, Heiman ML *et al*. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 1996;334:292–295.
41. Ostlund RE Jr, Yang JW, Klein S, Gingerich R. Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. *J Clin Endocrinol Metab* 1996;81:3909–3913.
42. Ravussin E, Bogardus C. Relationship of genetics, age, and physical fitness to daily energy expenditure and fuel utilization. *Am J Clin Nutr* 1989;49: 968–975.
43. Bandini LG, Schoeller DA, Dietz WH. Metabolic differences in response to a high-fat vs. a high-carbohydrate diet. *Obes Res* 1994;2:348–354.
44. Murphy P, Burnham WM. The ketogenic diet causes a reversible decrease in activity level in Long-Evans rats. *Exp Neurol* 2006;201:84–89.
45. Murphy P, Likhodii SS, Hatamian M, McIntyre Burnham W. Effect of the ketogenic diet on the activity level of Wistar rats. *Pediatr Res* 2005;57:353–357.
46. National Research Council. *Nutritional Requirements of Laboratory Animals*. National Academy of Sciences: Washington DC, 1995.
47. Evans SA, Messina MM, Knight WD, Parsons AD, Overton JM. Long-Evans and Sprague-Dawley rats exhibit divergent responses to refeeding after caloric restriction. *Am J Physiol Regul Integr Comp Physiol* 2005;288:R1468–R1476.
48. MacLean PS, Higgins JA, Johnson GC *et al*. Enhanced metabolic efficiency contributes to weight regain after weight loss in obesity-prone rats. *Am J Physiol Regul Integr Comp Physiol* 2004;287:R1306–R1315.
49. MacLean PS. A peripheral perspective of weight regain. *Am J Physiol Regul Integr Comp Physiol* 2005;288:R1447–R1449.