

Effect of Diet Composition on Leptin Concentration in Lean Subjects

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The recently discovered leptin is thought to be a satiety signal regulating food intake. In mice, it has been shown that on a high-fat diet leptin concentration increases, but the increase was explained by increased fat mass. It is yet unknown whether leptin is influenced by other nutritional factors. Here, leptin levels were measured in human volunteers on a high-fat diet, while maintaining energy balance. Twelve healthy, non-obese males and females (age, 26 ± 2 years; 21.4 ± 0.5 body mass index, habitual fat intake, 29 ± 1 energy % [en%]) consumed a high-fat diet (60 en% fat) for 7 days (days 1 to 7). Subjects were in energy balance (range, -0.15 to $+0.23$ MJ/d) as measured in a respiration chamber on days 1 to 3 and 7. Fasting baseline plasma leptin concentrations correlated with body fat percentage ($R^2 = .64$, $P < .005$). On average, no changes in leptin concentration on the high-fat diet were observed. However, on an individual basis, changes in leptin concentrations in response to the high-fat diet correlated with changes in insulin concentrations. In conclusion, in the case of energy balance, short-term changes in diet composition have no effect on fasting leptin concentration in lean subjects.

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HIGH-FAT DIETS often are considered to be fattening. Apart from a decreasing effect of the high-fat diet on energy expenditure (diet-induced thermogenesis), a higher energy intake while consuming high-fat diets has been observed.¹⁻³ This can be explained by the fact that people might eat to maintain carbohydrate stores,⁴ leading to overeating on a high-fat diet. However, it is still unclear whether people really eat to maintain carbohydrate stores.⁵⁻⁸ Another explanation for the hyperphagia and obesity seen on high-fat diets is the high energy density of such diets. A number of studies showed that subjects eat a similar weight of food irrespective of the diet composition, resulting in higher energy intake on energy-dense (high-fat) diets.^{3,9} However, although high-fat diets seem to result in a higher energy intake and obesity, in most people with a relatively stable body weight, fat balance over a lifetime is maintained within a 1% range.¹⁰ This observation has led to a lipostatic theory, first postulated in 1953 by Kennedy¹¹ and developed by Weigle.¹² Recently, discovery of the *ob* gene product leptin¹³ renewed interest in this theory. Leptin is produced by adipose tissue and is supposed to act as a satiety factor in regulating food intake. Administration of leptin decreased food intake and body weight in mice with a leptin deficiency due to mutation of the *ob* gene.¹⁴⁻¹⁷ In humans, leptin concentrations have been found to correlate well with body fat percentage.^{18,19} It is therefore suggested that obese subjects are resistant to leptin, possibly due to a defect in the leptin receptor.²⁰ Little is known about the factors that regulate or influence leptin concentrations in humans.

Frederich et al²¹ demonstrated that after 12 weeks' ad libitum consumption of a high-fat diet, leptin levels were significantly increased in mice. The increase in leptin levels could be explained by the increase in body fat content. Increased leptin levels did not result in reduced energy intake. One possibility therefore is that leptin plays no role in the defense against obesity induced by high-fat diets. However, it is also possible

that leptin does play a defendant role, but not sufficiently to reduce food intake and prevent obesity. In humans, no effects of high-fat diets on leptin have been reported so far.

We therefore measured leptin levels in human lean subjects given a high-fat diet for 1 week while being fed in energy balance. In this way, we could examine whether a high-fat diet, which normally leads to overeating, has an effect on leptin levels when no marked change in body weight and body composition occurs.

SUBJECTS AND METHODS

Subjects

Characteristics of the 12 volunteers (six men and six women) participating in this study are shown in Table 1. Women had significantly lower body weight ($P < .05$), greater body fat percentage ($P < .05$), and less fat-free mass ($P < .001$). No significant differences in age, body mass index, and fat mass were observed between men and women. All subjects were healthy and had a habitual diet that could be considered low-fat (mean \pm SEM energy intake, 9.0 ± 0.7 MJ, of which $29\% \pm 1\%$, $54\% \pm 2\%$, and $16\% \pm 1\%$ was provided as fat, carbohydrates, and proteins, respectively, as determined with a 3-day food intake record). All subjects were non-obese and did not have a family history of obesity. The study was approved by the Ethics Committee of the University of Limburg, and all subjects provided written informed consent.

Experimental Design

Subjects consumed a high-fat diet for 1 week (day 1 to day 7). For the first 3 days, as well as the last day, subjects stayed in the respiration chamber. On the other days, the high-fat diet was consumed at home. On the morning of day 4, subjects left the respiration chamber at 8 AM. They reentered the respiration chamber on the evening of day 6 for another 36-hour stay and left the chamber at 8 AM on day 8. Before the start of the high-fat diet, subjects were given a diet with the same composition as their habitual diet for 6 days. The last 2 days on the habitual diet, subjects stayed in the respiration chamber as part of another experiment on substrate utilization. Here, only data on the high-fat diet are presented.

Diets

All food was consumed as breakfast, lunch, dinner, and two or more snacks per day. The high-fat diet contained 60% of energy as fat, 25% of energy as carbohydrate, and 15% of energy as protein. Metabolizable energy intake and macronutrient composition of the diets were calculated using the Dutch food composition table.²² In the table, metabolizable energy is calculated by multiplying the amount of protein, fat, and

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Table 1. Subject Characteristics Measured at the End of the Experimental Period (mean \pm SEM)

Group	Age (yr)	Height (m)	Weight (kg)	Body Fat (%)	Body Mass Index (kg/m ²)
Total	26 \pm 2	1.77 \pm 0.03	67.0 \pm 2.4	19 \pm 2	21.4 \pm 0.5
Men	29 \pm 3	1.83 \pm 0.03	72.0 \pm 3.4	15 \pm 1	21.4 \pm 0.7
Women	23 \pm 1	1.71 \pm 0.03*	62.0 \pm 1.9*	23 \pm 2*	21.3 \pm 0.8

* $P < .05$ v men.

carbohydrate with the Atwater factors. For calculating macronutrient balances, the amount of protein, fat, and carbohydrate was multiplied by 0.909, 0.948, and 0.953, respectively, to correct for digestibility of macronutrients.

On the days spent at home, the high-fat diet was fully supplied to guarantee an unchanged macronutrient composition of the diet. The amount was based on the energy expenditure in the respiration chamber, plus free access to snacks with the same macronutrient composition. On the days spent in the respiration chamber, subjects were given an amount of energy equal to the 24-hour energy expenditure measured on the day preceding the high-fat diet. In this way, energy intake could be individually adjusted to energy expenditure, assuming that energy expenditure did not change during the experiment.

Procedures

Body composition. Subjects weighed themselves every morning during the stay in the respiration chamber, after voiding and before eating and drinking. Measurements were made on a digital balance (Seca delta, model 707, Hamburg, Germany) accurate to 0.1 kg. On the morning of day 8, body density was determined by underwater weighing in the fasted state, directly after subjects left the respiration chamber. Body weight was measured with a digital balance accurate to 0.01 kg (Sauter; type E1200, Albstadt-Ebingen, Germany). Lung volume was measured simultaneously with the helium dilution technique using a spirometer (Volugraph 2000; Mijnhardt, Bunnik, The Netherlands). Body fat percentage was calculated using the equations of Siri.²³ Fat-free mass in kilograms was calculated by subtracting fat mass from total body mass.

Indirect calorimetry and physical activity. Oxygen consumption and carbon dioxide production were measured in a whole-room indirect calorimeter, which was described previously.²⁴ Energy expenditure was calculated from O₂ consumption and CO₂ production according to the method of Weir.²⁵ In the respiration chamber, subjects followed an activity protocol consisting of fixed times for breakfast, lunch, and dinner, sedentary activities, and bench-stepping exercise. The bench-stepping exercise was performed for 30 minutes at intervals of 5 minutes' exercise alternated with 5 minutes' rest at a rate of 60 steps per minute with a bench height of 33 cm, repeated three times per day. Thus, subjects exercised for 45 minutes per day at a relatively low to medium intensity. At daytime, no sleeping or other exercise was allowed during the stay in the respiration chamber. Spontaneous physical activity of the subjects was monitored by means of a radar system based on the Doppler principle.

During the stay in the respiration chamber, 24-hour urine was collected from 8 AM to 8 AM. Subjects had to empty the bladder at 8 AM so that urine produced during the night could be included with the urine sample of the previous day. Samples were collected in containers with 10 mL H₂SO₄ to prevent nitrogen loss through evaporation; volume and nitrogen concentration were measured, the latter using a nitrogen analyzer (Heraeus; type CHN-O-Rapid, Hanau, Germany).

The 24-hour energy expenditure and 24-hour respiratory quotient were calculated from 8 AM to 8 AM. Carbohydrate, fat, and protein oxidation was calculated using O₂ consumption, CO₂ production, and urinary nitrogen loss with the equations of Brouwer.²⁶

Blood Analysis. Ten milliliters venous blood was sampled on the morning before entering the respiration chamber (baseline) and on the morning of days 4 and 8 after an overnight fast. Blood was collected in tubes containing EDTA to prevent clotting and immediately centrifuged at 3,000 rpm for 10 minutes. Plasma was frozen in liquid nitrogen and stored at -80°C until further analysis. Plasma substrates were determined using the hexokinase method (LaRoche, Basel, Switzerland) for glucose, the Wako NEFA C test kit (Wako Chemicals, Neuss, Germany) for free fatty acids (FFA), the glycerol kinase-lipase method (Boehringer, Mannheim, Germany) for glycerol and triglycerides, the ultrasensitive human insulin RIA kit (Linco Research, St Charles, MO) for insulin, and a radioimmunoassay (Linco Research) for leptin. Intraassay and interassay coefficients of variation for the leptin assay are less than 8%.

Statistical Analysis

All data are presented as the mean \pm SEM. Equality of energy intake and energy expenditure was determined by calculating the 95% confidence intervals for energy intake minus energy expenditure. The same method was used to determine equality of nutrient intake and oxidation. A one-way ANOVA with repeated measures was used to detect any differences in body weight or plasma substrates between days. When significant differences were found, a Tukey post hoc test was used to determine the exact location of this difference. Factorial ANOVA was used to examine all parameters for gender differences.

RESULTS

There were no significant differences in body weight between day 1 and day 3 (Table 2). A slight but significant decline in body weight of 0.4 ± 0.2 kg was observed between day 3 and day 7 (women 0.25 ± 0.30 kg v men 0.57 ± 0.27 kg, NS; Table 2, $P < .05$).

Energy balance was not significantly different from zero on days spent in the respiration chamber (Table 2). Protein balance was not significantly different from zero during the measurement days. On the high-fat diet, carbohydrate oxidation gradually declined ($P < .001$). This resulted in a significantly negative carbohydrate balance on days 1 to 3. On day 7, carbohydrate balance was not significantly different from zero (Table 3). Fat oxidation gradually increased on the high-fat diet ($P < .001$). On days 1 to 3, fat balance was significantly positive. On day 7, fat balance was not significantly different from zero (Table 3).

Blood Parameters

There were no significant differences in fasting leptin, glucose, and FFA concentrations between baseline, day 4, and day 8. In women, insulin was significantly lower on days 4 and 8 compared with baseline ($P < .05$; Table 4). Triglyceride concentration was significantly lower on day 4 compared with baseline in men, and glycerol concentration was significantly higher on day 4 compared with baseline in women ($P < .005$;

Table 2. Body Weight and Energy Balance Measured in the Respiration Chamber

Day	Body Weight (kg)	Energy Balance (MJ/d)
1	67.4 \pm 2.5*	-0.037 \pm 0.131
2	67.5 \pm 2.5*	0.068 \pm 0.116
3	67.3 \pm 2.5*	-0.150 \pm 0.176
7	66.9 \pm 2.4	0.226 \pm 0.134

* $P < .05$ v day 7.

Table 3. Substrate Balance Measured in the Respiration Chamber

Day	Carbohydrate Balance (g/d)	Fat Balance (g/d)	Protein Balance (g/d)
1	-72 ± 10*	27 ± 4*	8 ± 2
2	-45 ± 8*	19 ± 4*	3 ± 2
3	-33 ± 6*	14 ± 4*	-8 ± 3
7	-1 ± 10	6 ± 5	0 ± 3

* $P < .05$ v zero balance.

Table 4). Leptin concentration (day 8) was significantly correlated with body fat percentage ($R^2 = .64$, $P < .005$; Fig 1). Baseline leptin concentration correlated significantly with insulin concentration ($R^2 = .44$, $P < .05$). On days 4 and 8, leptin correlated significantly with triglyceride concentration ($R^2 = .45$, $P < .05$ and $R^2 = .52$, $P < .01$). On day 4 also, a significant correlation with glucose concentration ($R^2 = .48$, $P < .05$) was found. Changes in leptin concentration between day 4 and baseline correlated significantly with changes in FFA concentration ($R^2 = .41$, $P < .05$; Fig 2). Changes in leptin concentration between day 8, as well as day 4 and baseline correlated significantly with changes in insulin concentration between these days ($R^2 = .44$, $P < .05$, Fig 3; $R^2 = .45$, $P < .05$, Fig 4). Between day 4 and baseline, changes in FFA and insulin concentrations explained 56% of the variance in changes in leptin concentration ($P < .05$) as calculated with multiple regression analysis. Between day 8 and baseline, 61% of the variance in changes in leptin concentration was explained by changes in FFA, glycerol, and insulin concentrations ($P < .05$).

Leptin concentrations were significantly higher for women compared with men on all days ($P < .05$; Table 4). Triglycerides and FFA concentrations were significantly higher for women compared with men on day 4.

DISCUSSION

The results of the present study demonstrate that in situations where energy balance is maintained, changing the fat content of the diet has no influence on leptin concentrations over a period of 7 days.

In 1953, Kennedy¹¹ postulated that adipose tissue regulates its size by transmitting a satiety signal to feeding centers in the central nervous system. An increase in total adipose tissue mass leads to decreased appetite and decreased energy intake.¹² Since its discovery, leptin is thought to be this satiety signal. One prerequisite for leptin to be a satiety signal that regulates fat mass is a positive correlation between body fat percentage and leptin level. This has indeed been demonstrated in both mice and humans.^{18,19,27} In this study, we also observed a positive

correlation between leptin level and body fat percentage, even though the range of body fat percentage in our group of subjects was limited. It therefore seems that leptin levels reflect the amount of fat in the body.

The question remains as to whether leptin plays a defendant role in diet-induced obesity. High-fat foods often have been linked to obesity, both due to an impaired capacity of humans to oxidize fats (compared with carbohydrates) and/or due to a high energy density (and therefore higher energy intake) of such foods. Obesity seems to be a compensatory mechanism for a high dietary fat content. With increasing fat mass, fat-free mass will also be gained and energy expenditure will increase. Furthermore, it has been shown that increasing fat mass leads to higher rates of fat oxidation. It is therefore possible that an expansion of fat mass is necessary to maintain a stable body weight when high-fat diets are consumed, by (1) increasing fat oxidation and energy expenditure and (2) limiting a further increase in energy intake through elevated leptin levels.

When fed ad libitum, it has been shown that consumption of a high-fat diet leads to overeating and weight gain.¹⁻³ Frederich et al.²¹ demonstrated that consuming a high-fat diet increases leptin levels in mice. The observed increase in leptin was a reflection of the increase in adipose tissue stores. They therefore suggested that endogenous leptin, although increased by a high-fat diet, is not increased sufficiently to prevent the occurrence of diet-induced obesity. They also reported that despite the increased leptin levels, energy intake was not reduced compared with that of mice fed the standard diet.

When food intake is at energy balance, another mechanism is responsible for increasing fat oxidation when dietary fat content increases. Flatt⁴ proposed a model in which it is contended that when energy intake is restricted, lower carbohydrate stores have to be maintained to increase fat oxidation. In our study, subjects were fed in energy balance on a high-fat diet. This resulted in changes in nutrient oxidation, with a gradually increasing fat oxidation observed on the high-fat diet. Most likely this must have been due to decreasing carbohydrate stores, as indicated by the negative carbohydrate balance during days 1 to 3. During the first 3 days on the high-fat diet, subjects were in positive fat balance. This positive fat balance or changes in nutrient oxidation did not result in changes in leptin levels. It therefore appears that leptin does not respond to changes in nutrient oxidation or to an acute positive fat balance accompanied by a zero energy balance, at least in lean subjects. Possibly, greater changes in fat mass or changes in energy balance are necessary to produce any change in leptin levels. So within 7 days, diet composition seems to play no role in the production of leptin as

Table 4. Blood Parameters Measured Before, During, and After the High-Fat Diet

Group	Day	Glucose (mmol/L)	Triglycerides (μmol/L)	FFA (μmol/L)	Glycerol (μmol/L)	Insulin (μU/mL)	Leptin (ng/mL)
Women	Baseline	4.73 ± 0.23	908 ± 217	289 ± 54	54 ± 5	12.03 ± 2.35	12.72 ± 3.46*
	4	4.57 ± 0.06	666 ± 91*	386 ± 36*	86 ± 12†	8.16 ± 0.74†	10.24 ± 2.27*
	8	4.72 ± 0.10	703 ± 100	352 ± 31	73 ± 11	8.12 ± 1.09†	10.74 ± 2.83*
Men	Baseline	5.13 ± 0.34	721 ± 93	253 ± 35	61 ± 8	6.58 ± 1.84	3.33 ± 0.48
	4	4.80 ± 0.10	421 ± 40†	248 ± 26	59 ± 5	8.05 ± 1.06	3.47 ± 0.43
	8	4.86 ± 0.10	482 ± 40	263 ± 45	64 ± 6	7.08 ± 0.61	4.11 ± 0.43

* $P < .05$ v men.† $P < .05$ v baseline.

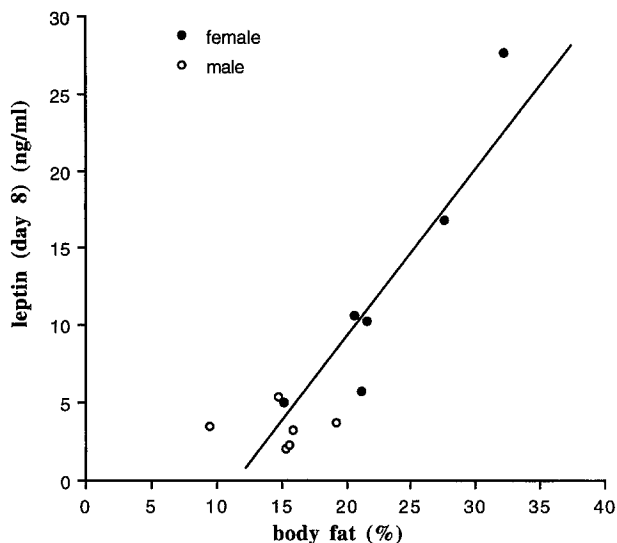


Fig 1. Relation between body fat percentage and fasting plasma leptin concentration.

long as body weight is maintained and energy balance is achieved. It has to be established whether this is also true for long-term adaptation to a high-fat diet.

In the current study, a decrease in body weight was observed after subjects left the respiration chamber (day 3 v day 7). It is possible that after a stay in the respiration chamber, subjects increased their physical activity without an accompanying increase in energy intake and therefore were in negative energy balance. However, the decrease in body weight might also be due to decreased glycogen concentrations. The small decrease in body weight might have had some influence on leptin levels. However, no correlation was found between changes in body weight and changes in leptin levels.

Considine et al¹⁹ showed that a 10% loss in body weight resulted in a 53% decrease in leptin concentration. Therefore,

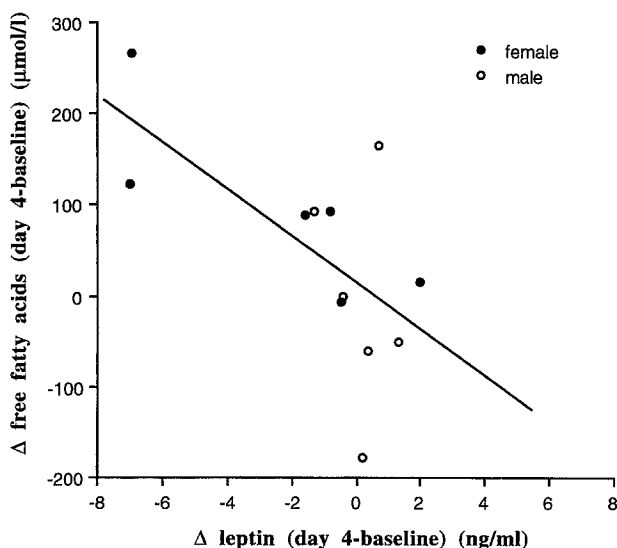


Fig 2. Relation between changes in FFA and leptin concentrations between day 4 and baseline.

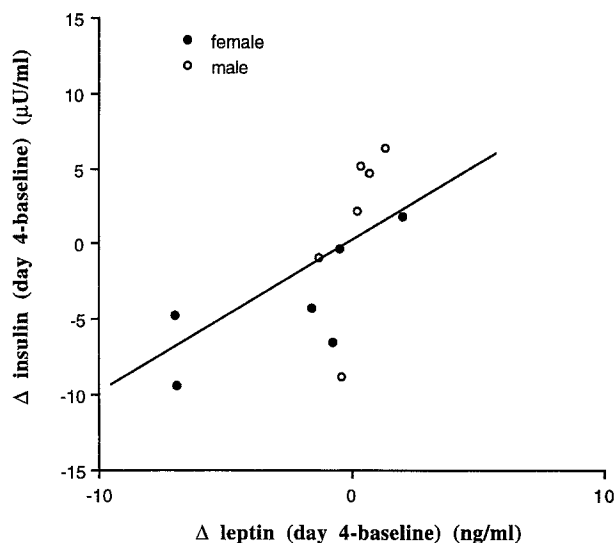


Fig 3. Relation between changes in insulin and leptin concentrations between day 4 and baseline.

they suggested that leptin concentrations are regulated by other factors in addition to adipose tissue size. One of the proposed factors is a declining fasting insulin concentration observed during the weight-loss period. Cusin et al²⁸ showed that in lean rats, insulin can be viewed as an upregulator and downregulator of *ob* expression. In our study, subjects were fed at energy balance consuming a high-fat diet. This resulted in a near-stable body weight, increased fat intake, and increased fat oxidation, and, on average, no effect on fasting leptin levels. However, on an individual basis, changes in leptin levels in response to altered fat content of the diet correlated with changes in insulin concentrations (and therefore with changes in FFA concentrations). It therefore seems that changes in diet composition, when accompanied by changes in insulin concentration (which might help to increase fat oxidation by regulating lipolysis), also lead to changes in leptin concentration. This indicates that

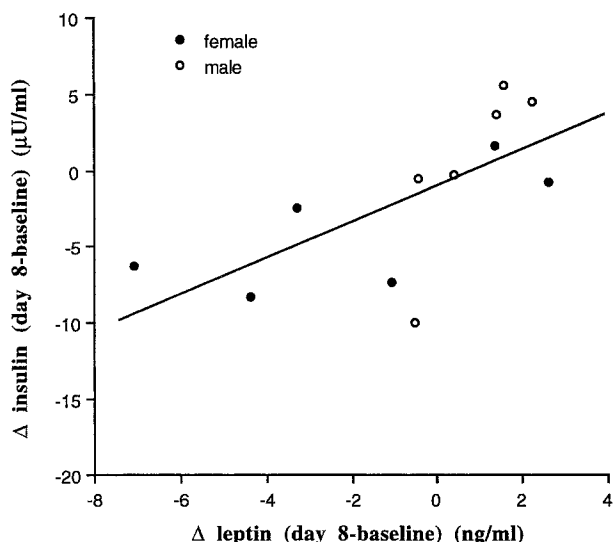


Fig 4. Relation between changes in insulin and leptin concentrations between day 8 and baseline.

insulin has a possible role in the upregulation and downregulation of leptin production, as suggested in an animal study.²⁸

Fasting leptin levels were closely correlated both with body fat percentage and with total body fat mass. The concentrations were threefold higher for women compared with men, as observed previously.^{18,19} In this study, fat mass and body mass index were not different between men and women. Therefore, the higher leptin concentrations in women were not explained by body mass index or fat mass, a finding also reported in mice,²¹ but might be explained by a higher body fat percentage in women. If leptin regulates the absolute amount of adipose tissue, then, most likely, women are less sensitive to leptin, because with equal amounts of fat mass more leptin is needed in

women than in men to maintain adipose tissue stores. Whether leptin plays an important role in the differences in body composition between women and men is yet unknown.

We conclude that in the case of near energy balance, changes in diet composition for a period of 7 days have no effect on fasting leptin concentration. So as long as energy balance is (nearly) maintained, changes in diet composition are not accompanied by changes in leptin, which might help to defend against the obesity often observed on high-fat diets. It is more likely that with a higher dietary fat content, elevation of the body's fat mass is necessary to maintain a stable body weight, by both elevated leptin levels and increased rates of fat oxidation and energy expenditure.

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