Lizards, Lipids, and Dietary Links to Animal Function

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ABSTRACT

Our experiments were designed to test the hypotheses that dietary lipids can affect whole-animal physiological processes in a manner concordant with changes in the fluidity of cell membranes. We measured (1) the lipid composition of five tissues, (2) body temperatures selected in a thermal gradient (T_{sel}), (3) the body temperature at which the righting reflex was lost (critical thermal minimal [CTMin]), and (4) resting metabolic rate (RMR) at three body temperatures in desert iguanas (*Dipsosaurus dorsalis*) fed diets enriched with either saturated or unsaturated fatty acids. The composition of lipids in tissues of the lizards generally reflected the lipids in their diets, but the particular classes and ratios of fatty acids varied among sampled organs, indicating the conservative nature of some tissues (e.g., brain) relative to others (e.g., depot fat). Lizards fed the diet enriched with saturated fatty acids selected warmer nighttime body temperatures than did lizards fed a diet enriched with unsaturated fatty acids. This difference is concordant with the hypothesis that the composition of dietary fats influences membrane fluidity and that ectotherms may compensate for such changes in fluidity by selecting different body temperatures. The CTMin of the two treatment groups was indistinguishable. This may reflect the conservatism of some tissues (e.g., brain) irrespective of diet treatment. The RMR of the saturated treatment group nearly doubled between 30° and 40°C. Here, some discrete membrane domains in the lizards fed the saturated diet may have been in a more-ordered phase at 30°C and then transformed to a less-ordered phase at 40°C. In contrast, the RMR of the unsaturated treatment group exhibited temperature independence in metabolic rate from 30° to 40°C. Perhaps the unsaturated diet resulted in membranes that developed a higher degree of disorder (i.e., a certain phase) at a lower temperature than were membranes of lizards fed the saturated diet. Our study demonstrates links between dietary fats and whole-animal physiology; however, the mechanistic basis of these links, and the general knowledge of lipid metabolism in squamate reptiles, remain poorly understood and warrant further study.

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Introduction

Diet composition can affect the lipid composition of biological membranes, tissues, and stored fat. For example, membranes with a high percentage of polyunsaturated fatty acids (PUFAs) appear to have greater fluidity relative to those with a high percentage of saturated fatty acids (SFAs; Hazel and Williams 1990). Additionally, stored fats that are rich in PUFAs can be more accessible as an energy source (Florant et al. 1993; Frank 1994; Frank and Storey 1995). In other words, dietary lipids appear to affect membrane and tissue-level lipid composition, which, in turn, affect whole-animal physiology and behavior. In one such case, after being fed a diet enriched with PUFAs, central netted dragons (*Ctenophorus* [*Amphibolurus*] *nuchalis*) incorporated more PUFAs in their adipose, liver, and muscle tissues than did lizards fed diets enriched with SFAs (Geiser and Learmonth 1994). These tissue-level changes were implicated in whole-animal responses in lizards (central netted dragons and Australian skinks [*Tiliqua rugosa*]), which selected cooler body temperatures when fed diets enriched with PUFAs relative to individuals fed diets high in SFAs (Geiser et al. 1992; Geiser and Learmonth 1994). Presumably, these changes in selected body temperature (T_{sel}) compensate for diet-induced changes in the fluidity of cell membranes, with cooler body temperatures decreasing fluidity, and warmer body temperatures increasing membrane fluidity. Some mammals also develop differences in the fatty acid composition of their tissues in response to diet, and these diet-induced differences have been shown to affect the duration of hibernation (Geiser 1991; Florant et al. 1993; Frank 1994; Frank and Storey 1995). Indeed, some mammalian hibernators preferentially forage on foods rich in PUFAs before hibernation. These PUFAs are selectively retained in depot fat before hibernation and are selectively metabolized during this period of dormancy (Florant et al. 1993;

Frank 1994). Thus, the data from both thermoregulating reptiles and hibernating mammals suggest an interplay between the diet-mediated lipid composition of certain tissues and body temperature.

However, the interactions among diet, membrane composition, tissue lipids, including depot fats, and whole-animal function (physiological and ecological) has not been the subject of any previous study. Indeed, some reports of whole-animal responses to differences in diet provide only correlative evidence because they do not examine the putative causal mechanisms (e.g., changes in membrane composition and fluidity).

We selected an herbivorous lizard, the desert iguana (*Dipsosaurus dorsalis*), because it has been well studied for whole-animal responses to thermal environments. For example, desert iguanas are thermophilic and thermoregulate with notable precision (Cowles and Bogert 1944; Norris 1953; DeWitt 1967), and previous studies have reported the metabolic rate of this species (e.g., Bennett and Dawson 1972). Additionally, as herbivores living in a seasonally variable environment (Norris 1953; Minnich and Shoemaker 1970; Krekorian 1976; Mautz and Nagy 1987), desert iguanas likely experience a variety of dietary lipids on a seasonal basis. Using the desert iguana, we connect the importance of dietary lipids to tissue and membrane composition, as well as whole-animal function for the first time in a reptile.

Our study had four objectives. First, we expanded on previous studies (e.g., Geiser et al. 1992; Geiser and Learmonth 1994) by separately analyzing the fatty acids of two major classes of lipids in the tissues of lizards to determine the extent to which the composition of either class was affected by diet. Specifically, we analyzed the phospholipid fraction—a major constituent of membranes—to understand the degree to which dietary fatty acids are incorporated into phospholipids. This analysis can be used to address more directly the hypotheses that alterations of membrane lipids are implicated in changes in whole-animal responses. We also analyzed the fatty acid content of triacylglycerol—a major energy storage molecule in adipose tissue—to determine whether the composition of this class of lipid is affected by diet. This analysis bears on the hypothesis that the degree of unsaturation of these molecules affects accessibility of stored energy, especially at low body temperatures. Second, we assessed T_{sel} in desert iguanas fed diets enriched with either saturated or polyunsaturated lipids to reevaluate the findings of Geiser et al. (1992) and Geiser and Learmonth (1994). Geiser and colleagues suggested that thermoregulating ectotherms may need to alter their body temperature to compensate for diet-induced changes in the fatty acid composition of cell and organelle membranes as well as tissues. Third, we determined the critical thermal minimum (CTMin) body temperature of desert iguanas to examine the extent to which diet-induced changes in the lipid composition of cellular membranes and/or depot fat alters ectotherm performance at low temperatures, as it has been shown to alter physiology in some mammals (Florant et al. 1993; Frank 1994). Last, we determined resting metabolic rate (RMR) to assess the extent to which diet-induced changes in the lipid composition of membranes results in changes in RMR, as predicted by Hulbert and Else (1999, 2000), who have suggested that membranes may function as "pacemakers of metabolism." These four experiments represent a comprehensive approach to the hypothesis that the lipid composition of an animal's diet can affect the composition of the tissues and membranes, which, in turn, can affect whole-animal physiology and behavior.

Material and Methods

Animals and Housing

Twenty-four adult male desert iguanas, *Dipsosaurus dorsalis* (mean mass = 74.4 g, range 52–109 g), were collected in and around the city of Twentynine Palms, California (34°09′N, 116°03′W) in the summer of 1994. The lizards were randomly assigned to one of two diet treatments: (1) a diet enriched with saturated fats or (2) a diet enriched with polyunsaturated fats (as described below). Animals were maintained in galvanized metal troughs (1.8 × 0.6 × 0.3 m) in groups of six (two groups per diet treatment). The floors of the troughs were covered with 2 cm of sand, and cement blocks and plastic tubes were provided for refugia. A 14L : 10D photoperiod was provided with both incandescent and full-spectrum fluorescent lights. Two 150-W heat lamps were placed overhead to provide a heterogeneous thermal environment (ca. 25°–65°C). Each trough also contained a 10 × 25-cm section of heat tape (Flexwatt, West Wareham, Mass.) placed under the sand that provided a constant (24-h) source of heat (to 45°C).

Lizard Diets and Diet Analyses

Experimental diets consisted of ground alfalfa-based Guinea pig chow (PMI Feeds, St. Louis) enriched with either 10% (by dry mass) hydrogenated coconut oil (melting point = 43°C; rich in palmitic acid [16:0]) for the saturated diet or 10% safflower oil (melting point ≈ −18°C; rich in linoleic acid [18:2]) for the polyunsaturated diet (ICN Biomedical, Cleveland). The enriched diets were mixed 1 : 4 (by mass) with deionized water. These diets were fed to the lizards ad lib. for at least 60 d before initiating experiments. Water was available ad lib. All animals remained in good health and most gained mass before the experiments.

Lipids were extracted from the diets for analysis using a modification (Reece et al. 1997) of the procedure described by Folch et al. (1957). Fatty acid composition was analyzed using standard gas chromatography techniques (e.g., Reece et al. 1997). Percentage composition of each fatty acid species was compared between diets ($N = 4$ subsamples per diet) with a *t*-test using SuperANOVA version 1.11 (Abacus Concepts 1989).

The saturated and unsaturated diets were significantly different in 10 of the 17 species of fatty acids measured (Table 1). As expected, the saturated diet contained proportionately more of the four shortest chain saturated fatty acids (8:0, 10:0, 12:0, 14:0), plus palmitic acid (16:0), and stearic acid (18:0). Likewise, the unsaturated diet was substantially higher in fatty acids that contained one or more double bonds, including a twofold increase in oleic acid (18:1) and an eightfold increase in linoleic acid (18:2). The fatty acid composition of a diet can also be expressed as the sum of the product of the number of double bonds and the percentage of each species of fatty acid contained in the diet. This value, termed the "unsaturation index" (UI), is commonly used to compare the relative unsaturation (or saturation) of experimental diets (e.g., Labbe et al. 1995). The UI calculated for the unsaturated diet used in our experiments was 4.5 times higher than the UI calculated for the saturated diet (Table 1). Further, the ratio of saturated fatty acids to unsaturated fatty acids (SFA/UFA) was approximately 18 times higher in the saturated diet than in the unsaturated diet.

Table 1: Fatty acid composition of the experimental diets

Fatty Acid	Saturated Diet	Unsaturated Diet	*P* Value
8:0	.36	.00	<.001*
10:0	2.01	.00	<.001*
12:0	31.62	.10	<.001*
14:0	15.16	.48	<.001*
14:1	.05	.06	1.000
15:0	.08	.09	.278
15:1	.02	.00	.356
16:0	14.70	10.93	<.001*
16:1	.54	.55	.670
17:0	.20	.19	.320
17:1	.11	.13	.171
18:0	14.28	4.87	<.001*
18:1	9.97	18.12	<.001*
18:2	8.06	60.35	<.001*
18:3	1.75	1.98	.017*
20:0	.26	.35	.002*
20:4	.07	.04	.478
UI	145.6	32.3	
SFA	78.6	17.0	
UFA	20.5	81.2	

Note. Values are means (%) based on four subsamples from each diet. UI = unsaturation index; SFA = saturated fatty acids; UFA = unsaturated fatty acids.

* Statistical difference between the diets is $P < 0.05$.

Fatty Acid Composition of Tissues

A sample of lizards from each group ($N = 3$ saturated; $N = 4$ unsaturated) was killed by decapitation immediately following 3 d in the T_{sel} experiments (described below). We harvested brain, heart, skeletal muscle (femoral), liver, and adipose tissues (abdominal fat bodies) from each animal. Tissue samples were blotted to remove excess moisture, weighed, immediately frozen with liquid nitrogen, and stored under N_2 at −80°C until the lipids were extracted.

We isolated total lipids from each sample using a chloroform : methanol phase separation extraction (Folch et al. 1957). Extracted lipids were dried under N_2. Samples were redissolved in an appropriate amount of $CHCl_3$ to yield an approximate concentration of 1 mg/mL (tissue sample/$CHCl_3$). Five microliters of 2% butylated hydroxytoluene (BHT) in CH_3OH per milliliter of extract was added to each sample to prevent oxidation. A sample (50 μL) of each was spotted on a silica gel thin-layer chromatography (TLC) plate. Triacylglycerol was separated from the phospholipids using a solvent system of petroleum ether : ether : acetic acid in a ratio of 90 : 12 : 1. The lipids were located by spraying the TLC plate with 2,7-dichloroflourescein (5 mmol/L in CH_3OH) and visualized with an ultraviolet light. A 19:0 internal fatty acid standard was added (200 nmol) to each triacylglyceride and phospholipid fraction for subsequent quantification. The lipids of interest were scraped from the TLC plates and collected into test tubes. Each sample was methylated by adding 2 mL of 14% BF_3 in CH_3OH, aerated with N_2, and heated at approximately 80°C for 15 min. The methyl esters were then extracted with 3 mL of petroleum ether. Five microliters of 2% BHT in CH_3OH per milliliter of extract was added again to each sample to prevent oxidation. A drying agent (sodium bicarbonate and anhydrous sodium sulfate) was added (2 : 1 by mass) to ensure solvent neutrality and to remove water. In preparation for the gas chromatography analyses, samples were dried under N_2 gas and redissolved in approximately 50 μL of CS_2. A 1-μL lipid sample was injected into a Shimadzu GC 14A gas chromatograph (Shimadzu Scientific Instruments, Columbia, Md.) with a SP-2340 capillary column and a fused silica column (30 m × 0.25 mm internal diameter; Supelco, Bellefonte, Pa.). Gas-flow conditions were as follows: air, 0.50 kg/cm^2; H_2, 0.60 kg/cm^2; He (carrier), 1.00 kg/cm^2; and He (makeup), 0.50 kg/cm^2. The following temperature program was used for the column: isothermal at 185°C for the first 6 min, followed by an increase of 10°C/min for the next 3 min and isothermal at 215°C for the last 7 min, for a total sample run time of 16 min. Injector and detector temperatures were maintained at 235°C.

Descriptive statistics were calculated using the EZChrom software package (Scientific Software 1995). We also compared both the percentage (of total) composition for each phospholipid species and several indices of lipid composition between the diet treatment groups with an ANOVA (Abacus Concepts 1989). Repeated-measures ANOVA was used to test for the effect of diet on the indices presented in Figures 1 and 2. If the overall effect of diet was significant between treatment groups, contrast analyses were performed to determine which

tissues differed significantly in these indices (Abacus Concepts 1989).

Selected Body Temperature Experiments

T_{sel}'s were measured in a thermal gradient (2.4 × 0.6 × 0.5 m) constructed of plywood with an aluminum floor covered by 2–3 cm of sand (as in Nussear et al. 1998). The gradient was divided in half, lengthwise, with a plywood partition so that the body temperature of two animals could be recorded simultaneously. One end of the gradient was heated via electrical resistance from the underside using three separately controlled wire-resistor circuits. Each circuit was connected to a rheostat that controlled the temperature for each of the three sections of the gradient. The opposite end of the gradient was cooled by chilled (4°–6°C) water and ethylene glycol (1 : 1 by volume) that was circulated continuously through copper coils fixed to the underside of the gradient floor. The gradient provided a range of substratum temperatures from 15°–65°C. A 14L : 10D photoperiod (0600–2000 hours) was provided by two full-spectrum fluorescent lights suspended 0.7 m above the gradient. A 4-W light 0.7 m above the gradient provided diffuse illumination and mitigated the abrupt darkness at the onset of nighttime.

We measured T_{sel} in 12 lizards fed the diet enriched with unsaturated fat and 12 lizards fed the diet rich in saturated fat. Following at least 60 d of acclimation to the lipid-enriched diets, one lizard from each treatment group was randomly selected and placed in one lane of the gradient. When in T_{sel} experiments, lizards were force-fed each morning at approximately 0800 hours by oral gavage in an amount that was determined to meet their energetic requirements (Nussear et al. 1998). Body temperatures were measured with a fine (38-ga) type-T thermocouple (Omega, Stamford, Conn.) inserted approximately 1.5 cm into the lizard's cloaca and secured to the tail with duct tape. The trailing thermocouple wire was inserted into fine polyethylene tubing, which reduced the likelihood of the lizards entangling themselves in the wire (Nussear et al. 1998). The thermocouple wires were suspended above each lane of the gradient on paper clips that traveled freely on a taut fine steel wire, thereby permitting the lizards to move unhindered. The animals were checked several times each day to ensure that the thermocouple wire did not become entangled or come out of the cloaca. Body temperatures were recorded at 5-min intervals for 3 d with either a CR-5 or CR-10 datalogger (Campbell Scientific, Logan, Utah).

The first day of the T_{sel} experiment was considered a period of acclimation to the gradient and, thus, was not included in the analyses. For days 2 and 3 of the experiment, the T_{sel} was averaged across 1000–1600 hours for each daytime and 2300–0500 hours for each nighttime period. These time periods were chosen a priori and reflect periods when the lizards were either active (daytime) or inactive (nighttime) and exclude times when lizards were manipulated or fed. As a result of entanglements or equipment errors, the entire 6-h daytime and 6-h nighttime T_{sel} periods were not recorded for all lizards on all days of the experiment. Therefore, we included in our analyses only lizards for which more than 80% of the data were recorded for a given period of each day. Further, lizards were not included in the analyses if their body mass changed by more than 5% during the 3 d of the experiment. Ultimately, the T_{sel} for 12 lizards fed the diet rich in unsaturated fats, and 10 lizards fed the diet rich in saturated fats were included in our analyses.

T_{sel}'s for each lizard during both daytime and nighttime were compared between treatment groups with a Mann-Whitney *U*-test using StatView (SAS Institute 1998) because of unequal variance between the treatment groups.

Critical Thermal Minimum

The CTMin body temperature is generally regarded as the temperature at which an animal ceases to function normally. In reptiles, this has been defined more precisely as the temperature at which the righting reflex is lost (Cowles and Bogert 1944; Spellerberg 1973). Measurements of CTMin were taken at the same time each day (1000–1300 hours) to avoid the potential effects of circadian rhythm on this response (Spellerberg and Hoffmann 1972). Lizards were housed and fed as described in the T_{sel} experiments. We determined CTMin in a manner similar to that described in Greer (1980). Lizards were placed individually into a glass aquarium, which was partially submerged in an ice bath. The aquarium floor was covered with a single layer of paper towels that were secured with tape. The towels reduced the rate of cooling and provided traction for righting. Body temperatures were monitored continuously with a 32-ga type-K thermocouple inserted approximately 1.5 cm into the cloaca and secured to the tail with tape. Lizards were slowly cooled (ca. 1°C/min) until they became sluggish (at ca. 15°C). The animals were then turned by their tails to a supine position and stroked on the abdomen with a fine brush. If the lizard righted itself, cooling was continued until its body temperature decreased another 1°C. This process was repeated until the lizard was barely able to right itself. Thereafter, we tested the righting reflex at 0.5°C intervals. CTMin was recorded as the temperature at which the lizard could no longer right itself after 30 s. At that point, the lizard was removed from the experimental chamber and placed in a heated aquarium to recover. Recovery was rapid, and long-term effects were not observed (as in Doughty 1994). We determined the CTMin twice for each lizard with a minimum 7-d recovery interval between measures. The CTMin data were analyzed with a repeated-measures ANOVA (Abacus Concepts 1989). The Pearson's correlation was calculated to determine repeatability of CTMin within individuals using StatView (SAS Institute 1998).

Resting Metabolic Rate

The same lizards that were used in the CTMin experiment were used to determine RMR. These lizards were fed the lipid-enriched diets for a minimum of 85 d before RMR was measured. We determined the RMR of lizards in each group using slight modifications of standard, closed-system respirometry techniques (Vleck 1987; see Nussear et al. 1998). This technique enabled us to obtain simultaneous measurements of a large number of lizards and circumvented the problem of measuring animals over different parts of their circadian cycle (Aschoff and Pohl 1970; Bennett 1972; Burggren 1997). Measurements were made between 1030–1530 hours, and after at least 24 h had elapsed since the last feeding.

Lizards were placed in 1,890-mL glass jars with wire-mesh lids in an environmental chamber where they were acclimated to the chamber temperature for at least 30 min. After acclimation, we replaced the screen lids with airtight lids fitted with inlet and outlet valves. After an additional 5 min, the air in the jars was mixed by pumping a 60-mL syringe connected to the inlet five times while both the inlet and outlet valves were open. Immediately after mixing, an initial air sample (60 mL) was collected, both valves were closed, and the time was recorded. The lizards remained in the jars for 30–90 min, depending on the temperature treatment (i.e., longer at lower temperatures). During this time, we observed the animals through a window at 1-min intervals to determine whether they remained at rest. Animals that moved during RMR measurements were excluded from the analyses for that temperature. After the appropriate time interval, the inlet tube was connected to a 60-mL syringe, its valve opened, and the gas in the jar mixed by repeatedly pumping the syringe (five times) with the outlet valve closed. Immediately after mixing, we collected a final 60-mL sample of gas and recorded the time.

Twelve lizards from each diet treatment were measured at three ecologically relevant temperatures (15°, 30°, and 40° ± 1°C). Oxygen consumption was measured three times at 30° and 40°C over a period of 9 d. As a result of lizards moving during RMR measurements, sample sizes varied from nine to 10 individuals per treatment group per measurement period. The lowest measured RMR for each lizard for the three measurement periods and for a given temperature was used in subsequent analyses to avoid including individuals with unobserved movements. We measured RMR a single time at 15°C because no animals were observed moving at this temperature.

Oxygen concentration of the air samples was determined with an Ametek S-3A/II dual-channel oxygen analyzer that was operated in differential mode. The analyzer was plumbed as follows. Starting from the upstream end, there was a long piece (ca. 1.5 m) of narrow plastic tubing open to room air. The downstream end of the tubing was connected to a small tube containing Drierite, Ascarite, then more Drierite (to remove water and carbon dioxide, respectively). Downstream of the Drierite and Ascarite, the tubing was connected to the inlet port of the first channel of the oxygen analyzer. Air was pulled through the sensor with a downstream precision-flow controller at 95 mL/min. An identical setup was connected to the second channel of the oxygen analyzer. Upstream of the Drierite and Ascarite in the second channel, an 18-ga needle was inserted pointing upstream. The connection of the needle to room air was closed with a two-way valve. Gas samples were injected into the tubing leading to sensor (channel) two of the analyzer. The sample was injected by hand at a faster rate (i.e., 120 mL/min) than the air was drawn into the sensor by the downstream pump. The tubing was open on the upstream end to prevent the induction of a pressure change that would affect the reading obtained on this type of oxygen analyzer. Because the rate of injection of the gas sample (120 mL/min) exceeded the rate at which air was drawn from the tube into the analyzer (95 mL/min), the sample was injected as a single bolus of gas that was then drawn into the sensor.

We used DataCan V software (Sable Systems 1992) to record the difference in oxygen concentration between the control channel and the air sampled from the jars containing the lizards. Samples were recorded at 0.5-s intervals. Injected air samples typically resulted in a rapid rise in differential oxygen concentration, followed by a plateau, and then a rapid return to ambient oxygen concentrations. The differential oxygen concentration was then subtracted from 0.2095 to obtain the actual oxygen concentration. Rate of oxygen consumption was calculated according to Vleck (1987, eq. [9]). We estimated the volume of air in the jars by subtracting the volume displaced by each lizard (assuming 0.98 g = 1 mL; De Vera and Hayes 1995) from the volume of the jar. We assumed that the initial fractional concentrations of water vapor and carbon dioxide in the jars were zero (Vleck 1987). These assumptions are not strictly valid, but analyses of initial gas concentrations (sensu Vleck 1987) indicate that this method only slightly overestimates oxygen consumption (mean + 0.5%; maximum error + 1.1%; Nussear et al. 1998). All estimates of oxygen consumption were corrected to standard temperature and pressure (0°C; 101325 Pa) and are reported as milliliters of O_2 per hour.

Some lizards were excluded from the analyses because they moved during the RMR measurements. Therefore, actual sample sizes used in the RMR analyses were as follows: 15°C saturated = 10, unsaturated = 9; 30°C saturated = 9, unsaturated = 10; 40°C saturated = 10, unsaturated = 10. Mean mass of the lizards used in each trial were as follows: at 15°C unsaturated = 54.2 g, saturated = 51.1 g; at 30°C unsaturated = 56.2 g, saturated = 53.3 g; at 40°C unsaturated = 56.2 g, saturated = 52.9 g. We compared RMRs between the unsaturated and saturated treatment groups using a repeated-measures ANCOVA (Abacus Concepts 1989), where diet was the treatment and body mass was the covariate. Mass-specific values were not used to avoid the potential errors in both

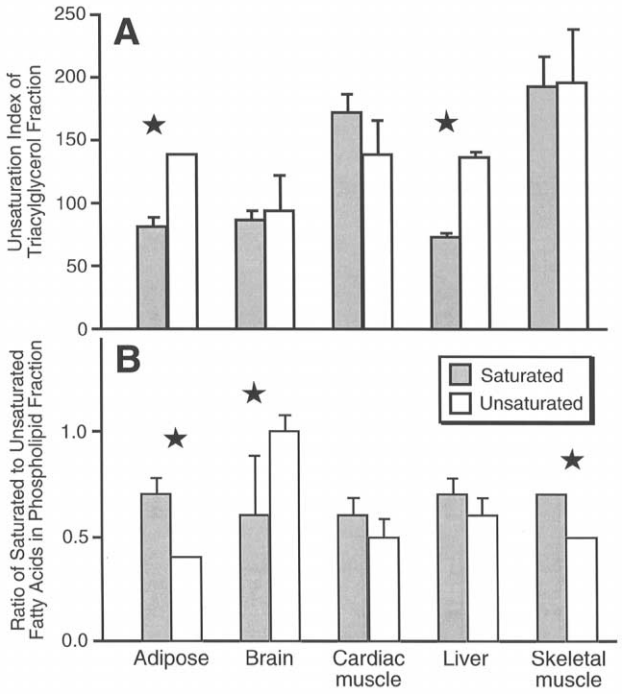

Figure 1. *A*, Comparisons of the unsaturation index for triacylglycerol (a correlate of fluidity of stored fat) for desert iguanas fed diets rich in saturated or unsaturated fats (mean + SD). *B*, Comparisons of the ratio of saturated to unsaturated fatty acids in the phospholipid fraction (a correlate of membrane fluidity). Histograms lacking error bars have very small standard deviations. Stars indicate statistical differences ($P < 0.05$) within tissues.

statistical analysis and interpretation that are possible when ratios are used to attempt to remove the effect body mass (see Hayes and Shonkwiler 1996; Packard and Boardman 1999). Contrast analyses were performed to test for statistical differences within and among the experimental temperatures (Abacus Concepts 1989).

Results

Fatty Acids in Tissues

Results of the fatty acid analyses from the five tissues sampled from desert iguanas are provided in Tables 2–6. In general, treatment groups differed significantly in the fatty acid content of triacylglycerol in their tissues for those species of fatty acid that were dramatically different in the corresponding diet (Fig. 1*A*), whereas there was not a strong affect of the dietary treatments on the phospholipid fraction from the same tissues (Fig. 1*B*). Across all tissues and between diet treatments, fatty acids tended to be more similar to the diet in the triacylglycerol fraction than in the phospholipid fraction. The UI (a correlate of fluidity in stored fats; Frank 1994) of triacylglycerol was significantly lower in both adipose and liver tissue (Fig. 2*A*). The SFA/UFA ratio (a known correlate of membrane fluidity; Hazel and Williams 1990) of the phospholipid fraction was significantly higher in lizards fed the saturated diet in both adipose tissue and skeletal muscle, and significantly lower in brain tissue (Fig. 2*B*).

Adipose tissue was the most affected by the diet treatments. Most species of fatty acids that differed in the diets, and those species that were subsequently synthesized from these dietary precursors and incorporated into the fat bodies, were significantly different in the triacylglycerol fraction (Table 2). In addition, nearly all saturation indices indicated a 0.15- to nearly fivefold differences among lipids in both the phospholipid and triacylglycerol fractions of adipose tissues (Table 2). Although brain tissue exhibited differences in three phospholipid and five triacylglycerol fatty acid species, the various saturation indices indicated only a small difference in the n6/n3 ratios of the phospholipid fraction and in one group of fatty acids (MUFA) in the triacylglycerol fraction (Table 3). Cardiac muscle had five differences in both the phospholipid and triacylglycerol frac-

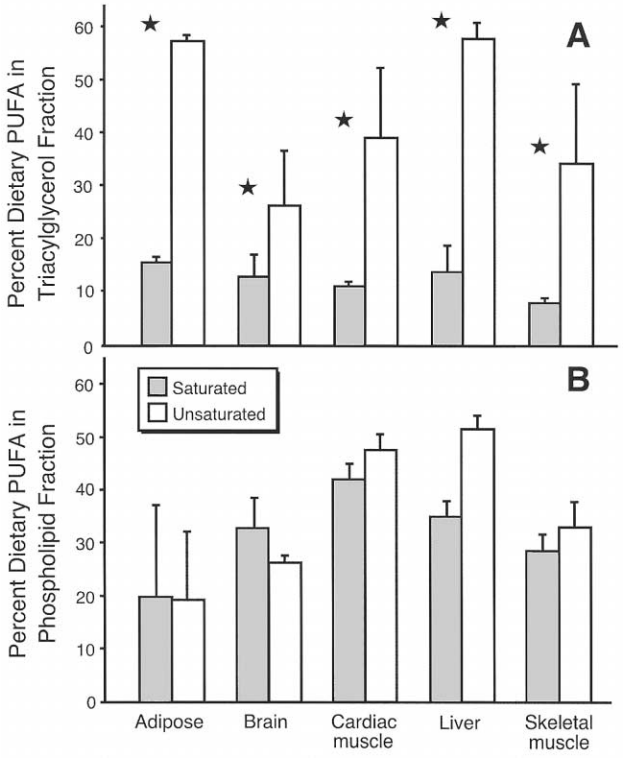

Figure 2. *A*, Comparisons of the percentage (mean + SD) of polyunsaturated fatty acid (*PUFA*) that may have originated from the diets in desert iguanas were universally different between diet treatments. Stars indicate statistical differences ($P < 0.05$) within tissues. *B*, In contrast, the same comparisons for the phospholipid fractions were not different between diet treatments.

Table 2: Summary of fatty acid composition (%) of adipose tissue phospholipids and triacylglycerol of desert iguanas fed diets rich in saturated and unsaturated fats

Fatty Acid Species	Phospholipids		Triacylglycerol	
	Saturated Diet ($N = 2$)	Unsaturated Diet ($N = 4$)	Saturated Diet ($N = 2$)	Unsaturated Diet ($N = 4$)
15:0	7.16 (5.30)	12.65 (4.97)	1.80 (.59)	.30 (.03)*
16:0	19.91 (3.44)	7.96 (3.99)*	24.78 (3.42)	14.63 (1.10)*
16:1	4.04 (5.71)	9.81 (2.97)	6.89 (3.57)	1.39 (.16)*
18:0	10.77 (8.46)	4.97 (2.29)	7.38 (3.66)	4.51 (.11)
18:1n-9	12.33 (7.72)	8.18 (5.16)	40.73 (.34)	18.92 (.15)*
18:2n-6	14.57 (11.95)	17.00 (12.90)	15.11 (1.50)	57.00 (1.54)*
20:0	1.38 (1.95)	3.66 (1.17)	.25 (.05)	.21 (.04)
20:1	4.50 (2.68)	6.46 (1.93)	2.41 (.88)	1.92 (.17)
20:2	6.22 (.30)	1.29 (.96)*	.13 (.05)	.33 (.04)*
20:3n-6	1.00 (1.41)	1.82 (1.43)	.10 (.00)	.05 (.06)
20:4n-6	4.84 (5.57)	1.85 (.84)	.10 (.04)	.12 (.08)
20:5n-3	.31 (.44)	.39 (.45)	.02 (.02)	.06 (.04)
22:4n-6	.66 (.93)	1.29 (1.02)	.05 (.02)	.01 (.03)
22:5n-6	1.25 (1.76)	2.43 (1.79)	.02 (.03)	.06 (.05)
22:5n-3	.17 (.23)	.00 (.00)	—	—
22:6n-3	10.39 (8.78)	20.17 (8.7)	.2 (.2)	.5 (.3)
UI	158.3 (27.9)	214.1 (42.8)	83.0 (9.3)	141.1 (2.6)*
SFA	39.2 (2.2)	29.2 (1.1)*	34.2 (6.5)	19.6 (1.1)*
UFA	60.2 (3.0)	70.7 (1.0)*	65.8 (6.5)	80.4 (1.1)*
SFA/UFA	.7 (.1)	.4 (.0)*	.5 (.2)	.3 (.0)*
PUFA	39.4 (3.7)	46.2 (.6)*	15.8 (1.7)	58.1 (1.4)*
PUFA Diet	19.4 (17.5)	18.9 (13.4)	15.2 (1.5)	57.1 (1.5)*
PUFA >3 db	17.6 (6.6)	26.1 (11.0)	.4 (.3)	.8 (.5)
MUFA	20.9 (.7)	24.5 (1.2)*	50.0 (4.8)	22.2 (.4)*
n6	22.3 (13.4)	24.4 (9.6)	15.4 (1.6)	57.2 (1.6)*
n3	10.9 (9.5)	20.6 (9.0)	.3 (.2)	.6 (.4)
n6/n3	4.2 (4.9)	1.9 (2.3)	103.5 (91.6)	150.4 (98.2)

Note. Data are mean percentages (±SD). UI = unsaturation index; SFA = saturated fatty acids; UFA = unsaturated fatty acids; PUFA = polyunsaturated fatty acids; PUFA diet = PUFA that could have originated from the diet; PUFA >3 db = PUFAs containing more than three double bonds; MUFA = monounsaturated fatty acids; n6 = omega-6 fatty acid; n3 = omega-3 fatty acid. Dashes indicate that a fatty acid species was undetected in both treatment groups.

* Significant differences in tissue compositions between diet treatments at $P \leq 0.05$.

tions (Table 4). The most notable among these were two essential fatty acids: oleic and linoleic acid (18:1 and 18:2, respectively). Like depot fat, liver tissues exhibited significant differences between diet treatments in most of the species of fatty acids that either originated (four of five), or were synthesized from their respective diets. There were more differences in fatty acid species and various saturation indices in the triacylglycerol fraction than in the phospholipid fraction of liver as well (Table 5). Skeletal muscle had only three differences in fatty acid species in both fractions, but these differences were of sufficient magnitude that they translated into significant differences among three indices of saturation in the phospholipid fraction, and five differences among the saturation indices in the triacylglycerol fraction (Table 6).

Selected Body Temperatures

Daytime T_{sel} (38.3° ± 0.9°C [mean ± SD], $N = 9$ [sample size]) was not significantly different from nighttime T_{sel} (37.6° ± 2.2°C, $N = 9$; $U = 48$, $P = 0.807$) for lizards fed the diet rich in saturated fats, whereas daytime T_{sel} (38.3° ± 0.9°C, $N = 12$) was significantly different from nighttime T_{sel} (34.2° ± 5.2°C, $N = 12$) in the lizards fed a diet rich in unsaturated fats ($U = 128$, $P = 0.001$; Fig. 1). Furthermore, the nighttime body temperatures for lizards fed the diet rich in unsaturated fats were cooler than those selected by lizards fed the diet rich in saturated fats ($U = 89.0$, $P = 0.056$; Fig. 3). These results are influenced by two individuals that selected especially low nighttime body temperatures (17.9° and 20.6°C)

Table 3: Summary of fatty acid composition (%) of brain tissue phospholipids and triacylglycerol of desert iguanas fed diets rich in saturated and unsaturated fats

Fatty Acid Species	Phospholipids		Triacylglycerol	
	Saturated Diet ($N = 3$)	Unsaturated Diet ($N = 4$)	Saturated Diet ($N = 3$)	Unsaturated Diet ($N = 4$)
15:0	5.84 (2.56)	7.30 (.47)	.78 (.70)	.51 (1.02)
16:0	24.15 (10.24)	35.90 (2.66)	25.80 (11.52)	27.05 (3.88)
16:1	1.73 (.99)	1.89 (.53)	5.05 (.96)	1.08 (2.16)*
18:0	4.65 (1.57)	2.93 (.32)	6.12 (1.13)	12.49 (3.58)*
18:1n-9	15.32 (6.95)	13.98 (3.30)	45.50 (6.70)	28.45 (2.93)*
18:2n-6	22.03 (4.49)	16.06 (1.34)*	12.22 (4.45)	23.03 (11.44)
20:0	1.76 (.60)	2.55 (.11)*	.23 (.20)	.22 (.27)
20:1	.30 (.04)	.39 (.33)	2.07 (.22)	1.40 (.39)*
20:2	2.19 (1.62)	.80 (.43)	.08 (.13)	.26 (.33)
20:3n-6	.66 (.19)	.57 (.08)	.04 (.08)	.14 (.28)
20:4n-6	10.47 (2.65)	9.94 (.79)	.28 (.25)	3.11 (1.46)*
20:5n-3	.23 (.09)	.05 (.06)*	.05 (.05)	.42 (.49)
22:4n-6	2.57 (1.18)	2.00 (.15)	.73 (1.05)	.31 (.62)
22:5n-6	1.06 (.84)	.51 (.05)	.32 (.55)	.10 (.20)
22:5n-3	.19 (.14)	.21 (.04)	—	—
22:6n-3	6.86 (2.03)	4.94 (.27)	.73 (.84)	1.43 (.61)
UI	168.5 (38.1)	132.9 (5.3)	87.5 (11.0)	95.8 (29.2)
SFA	36.4 (9.7)	48.7 (2.7)	32.9 (10.7)	40.3 (7.7)
UFA	63.6 (9.7)	51.3 (2.7)	67.1 (10.7)	59.7 (7.7)
SFA/UFA	.6 (.3)	1.0 (.1)	.5 (.2)	.7 (.2)
PUFA	46.3 (11.7)	35.1 (2.0)	14.5 (2.9)	28.8 (10.0)
PUFA Diet	32.5 (6.1)	26.0 (1.7)	12.5 (4.7)	26.1 (10.6)
PUFA >3 db	21.4 (6.2)	17.6 (1.1)	2.1 (2.2)	5.4 (2.7)
MUFA	17.3 (6.1)	16.3 (3.2)	52.6 (7.8)	30.9 (4.5)*
n6	14.8 (4.3)	13.0 (.9)	14.0 (3.1)	27.9 (10.1)
n3	7.3 (2.1)	5.2 (.2)	.8 (.8)	1.9 (0.8)
n6/n3	2.0 (.0)	2.5 (.1)*	33.5 (23.8)	22.3 (23.6)

Note. Data are mean percentages (±SD). Abbreviations follow Table 2.

* Significant differences in tissue compositions between diet treatments at $P \leq 0.05$.

on one of the two nights they were in the gradient. Daytime T_{sel} did not differ significantly between treatment groups ($U = 66.0$, $P = 0.394$).

Critical Thermal Minimum Body Temperature

The CTMin of desert iguanas fed a diet enriched in saturated ($N = 12$) or unsaturated ($N = 14$) fats was 13.8° ± 1.2°C (mean ± SD) and 14.2° ± 1.0°C, respectively. These temperatures were statistically indistinguishable ($t_{1,25} = 0.952$, $P = 0.34$). A Pearson's correlation indicated that CTMin, measured 7 d apart, was repeatable for individual lizards ($r = 0.513$, $P = 0.007$).

Resting Metabolic Rate

Overall, there was no significant effect of diet on RMR across the three experimental temperatures ($P = 0.49$). However, the ANCOVA revealed a significant interaction ($P = 0.004$) between diet and RMR measured across the three experimental temperatures. This interaction resulted from a nearly twofold increase ($P < 0.0001$) in RMR measured from 30° to 40°C for lizards fed the saturated fat diet and the lack of a significant difference ($P = 0.12$) in RMR for lizards fed the unsaturated diet over the same two temperatures (Fig. 4). The RMR of lizards in the unsaturated treatment group was significantly higher than the RMR of lizards in the saturated treatment group at 30°C ($P = 0.01$) but did not differ when measured at either 15° or 40°C ($P = 0.18$ and $P = 0.68$, respectively; Fig. 4). The ANCOVA also revealed a significant interaction of body mass with RMR at the experimental temperatures ($P = 0.007$). In-

Table 4: Summary of fatty acid composition (%) of cardiac muscle phospholipids and triacylglycerol of desert iguanas fed diets rich in saturated and unsaturated fats

Fatty Acid Species	Phospholipids		Triacylglycerol	
	Saturated Diet ($N = 3$)	Unsaturated Diet ($N = 3$)	Saturated Diet ($N = 3$)	Unsaturated Diet ($N = 3$)
15:0	5.06 (.79)	3.85 (.38)	9.10 (2.21)	2.61 (2.94)*
16:0	10.88 (1.30)	8.37 (1.03)	16.12 (5.41)	17.18 (4.63)
16:1	2.43 (.03)	2.54 (.58)	6.69 (3.12)	2.06 (2.04)
18:0	20.11 (1.60)	20.61 (2.52)	5.45 (1.92)	6.87 (1.31)
18:1n-9	8.17 (.69)	5.04 (.71)*	16.56 (8.11)	21.00 (8.85)
18:2n-6	29.25 (2.35)	37.74 (3.80)*	8.26 (2.56)	37.78 (13.54)*
20:0	1.60 (1.08)	.97 (.60)	5.82 (3.85)	1.22 (.30)
20:1	2.96 (1.15)	1.80 (1.17)	6.20 (.23)	3.22 (1.77)*
20:2	.42 (.13)	2.41 (.81)*	2.61 (3.17)	1.24 (.81)
20:3n-6	.77 (.40)	.87 (.52)	2.24 (.66)	.67 (.72)*
20:4n-6	12.50 (1.05)	9.57 (1.02)*	2.39 (1.42)	1.16 (.63)
20:5n-3	.56 (.18)	.23 (.21)	1.13 (.81)	.18 (.16)
22:4n-6	.60 (.14)	.56 (.16)	1.01 (.68)	.36 (.31)
22:5n-6	.51 (.28)	.54 (.40)	1.60 (.46)	.54 (.67)
22:5n-3	.40 (.14)	.37 (.42)	.67 (.78)	.12 (.15)
22:6n-3	3.78 (2.79)	4.54 (4.11)	14.16 (1.93)	3.79 (4.81)*
UI	157.6 (14.0)	165.8 (25.5)	173.4 (17.5)	139.4 (29.5)
SFA	37.6 (2.4)	33.8 (2.8)	36.5 (3.6)	27.9 (5.1)
UFA	62.4 (2.4)	66.2 (2.8)	63.5 (3.6)	72.1 (5.1)
SFA/UFA	.6 (.1)	.5 (.1)	.6 (.1)	.4 (.1)
PUFA	48.8 (2.0)	56.8 (2.6)*	34.1 (4.9)	45.8 (12.4)
PUFA Diet	41.7 (3.2)	47.3 (3.3)	10.7 (1.2)	38.9 (13.5)*
PUFA >3 db	18.3 (2.2)	15.8 (5.5)	21.0 (4.1)	6.2 (6.1)*
MUFA	13.6 (1.0)	9.4 (.6)*	29.5 (5.6)	26.3 (7.6)
n6	43.6 (2.6)	49.3 (2.6)	7.2 (3.0)	2.7 (2.3)
n3	4.7 (2.9)	5.1 (4.6)	16.0 (1.8)	4.1 (4.6)*
n6/n3	14.8 (14.1)	6.3 (1.1)	.4 (.1)	1.3 (1.2)

Note. Data are mean percentages (±SD). Abbreviations follow Table 2.

* Significant differences in tissue compositions between diet treatments at $P \leq 0.05$.

spection of the analysis suggests that this resulted from a slight, but significant, effect of body mass on RMR at 15° and 30°C but no significant effect at 40°C.

Discussion

Our research demonstrates that alterations of dietary lipids can result in concomitant alterations of the lipid composition of important tissues. The alteration of the lipid composition of tissues is implicated as the causal mechanism for the observed differences in behavioral thermoregulation and alterations of metabolic rates under some conditions in these lizards, thereby suggesting a general relationship between lipid composition of tissues and whole-animal responses. Below, we review the potential mechanisms that relate lipid composition to whole-animal function and show how these mechanisms may bear on our results.

Known and Hypothesized Effects of Diet and Temperature on Membrane Structure and Function

The physical structure of lipids in biological membranes or tissues can alter important aspects of cellular- and tissue-level processes. For example, the fluidity of the cell membrane is affected, in part, by changes in its lipid composition. Membrane fluidity is considered to be important because of the critical role cell membranes play in processes such as diffusion across the membrane and in storing and regulating energy in the form of transmembrane ion gradients (Hazel and Williams 1990). These processes may be disrupted by changes in membrane fluidity (Hazel and Williams 1990). In biological membranes, the presence of unsaturated fatty acids tends to increase membrane fluidity. This is because the carbon-carbon double bonds—which form the hydrocarbon chains or "tails" of unsaturated fatty acids—do not allow membrane phospholipids

Table 5: Summary of fatty acid composition (%) of liver tissue phospholipids and triacylglycerol of desert iguanas fed diets rich in saturated and unsaturated fats

Fatty Acid Species	Phospholipids		Triacylglycerol	
	Saturated Diet ($N = 3$)	Unsaturated Diet ($N = 4$)	Saturated Diet ($N = 3$)	Unsaturated Diet ($N = 4$)
15:0	1.34 (1.16)	1.40 (.27)	1.22 (2.12)	.22 (.25)
16:0	19.48 (2.19)	14.93 (1.78)*	30.00 (.84)	16.28 (2.30)*
16:1	3.67 (1.62)	.45 (.30)	9.01 (6.08)	.70 (.58)*
18:0	19.71 (1.07)	21.08 (5.93)	7.78 (2.90)	4.73 (.52)
18:1n-9	15.91 (4.22)	8.80 (1.13)*	36.01 (1.87)	18.01 (2.01)*
18:2n-6	29.41 (3.14)	45.98 (3.00)*	13.35 (5.26)	57.36 (3.63)*
20:0	.46 (.40)	.44 (.06)	.21 (.05)	.15 (.06)
20:1	1.56 (1.35)	.75 (.13)	2.25 (.82)	1.47 (.23)
20:2	1.07 (1.18)	.81 (.28)	.00 (.00)	.63 (.24)*
20:3n-6	.73 (1.07)	.16 (.13)	.00 (.00)	.17 (.03)*
20:3n-3	.00 (.00)	.06 (.11)	.00 (.00)	.01 (.02)
20:4n-6	5.19 (.25)	5.16 (.29)	.18 (.19)	.29 (.02)
22:6n-3	1.97 (2.26)	.00 (.00)	—	—
UI	116.8 (17.9)	124.9 (7.0)	74.7 (5.8)	137.9 (5.9)*
SFA	41.0 (2.3)	37.8 (4.0)	39.2 (.2)	21.4 (2.4)*
UFA	59.5 (1.8)	62.2 (4.0)	60.8 (.2)	78.6 (2.4)*
SFA/UFA	.7 (.1)	.6 (.1)	.6 (.0)	.3 (.0)*
PUFA	38.4 (6.0)	52.2 (3.0)*	13.5 (5.4)	58.5 (3.5)*
PUFA Diet	34.6 (3.4)	51.1 (3.0)*	13.5 (5.4)	57.6 (3.6)*
PUFA >3 db	7.2 (2.5)	5.2 (.3)	.2 (.2)	.3 (.0)
MUFA	21.1 (4.3)	10.0 (1.3)*	47.3 (5.4)	20.2 (1.3)*
n6	35.3 (4.4)	51.3 (3.0)*	13.5 (5.4)	57.8 (3.6)*
n3	2.0 (2.3)	.1 (.1)	.0 (.0)	.0 (.0)

Note. Data are mean percentages (±SD). Abbreviations follow Table 2.

* Significant differences in tissue compositions between diet treatments at $P \leq 0.05$.

to pack as tightly as saturated phospholipids, which lack such double bonds (Hazel and Williams 1990).

Another factor that influences membrane fluidity is temperature. An increase in body temperature will increase membrane fluidity (Hochachka and Somero 1984; Hazel and Williams 1990; Gibbs 1998). Regulation of membrane fluidity at different temperatures via control of membrane-lipid composition has been termed "homeoviscous adaptation" (HVA; Sinensky 1974; Cossins 1994; Hazel 1995; Williams 1998). Examples of HVA are pervasive in organisms that are experimentally acclimated to different temperatures. In fact, HVA of membranes to changes in temperature has been demonstrated in every taxon that has been studied to date (Hazel and Williams 1990; Gibbs 1998). Regulation of the proportion of saturated fatty acids in the membrane is a common response to changes in temperature and the ratio of saturated to unsaturated fatty acids (SFA/UFA) is known to correlate with membrane fluidity (Hazel and Williams 1990). Unfortunately, the ramifications of these cellular modifications at the level of the whole animal are poorly understood (Gibbs 1998).

In addition to HVA—wherein cells alter the lipid composition of their membranes as a compensatory response to thermal challenges—animals may also adjust their body temperature, with lower body temperature decreasing fluidity and higher body temperature increasing fluidity, to compensate for changes in membrane composition. Therefore, thermoregulating ectotherms may be able to compensate for changes in the diet-induced lipid composition of the membranes by altering their body temperature (Geiser et al. 1992; Geiser and Learmonth 1994).

A less frequently cited alternative hypothesis concerning the response of membranes to both temperature and fluidity has recently regained interest among some physiologists (Hazel et al. 1998; Williams 1998). This hypothesis, termed "homeophasic adaptation," suggests that, although both temperature and the degree of saturation of the constituent lipids are important, biological membranes exist in distinct physical phases that can be described by their degree of order, and these phases play an important role in determining membrane structure and function (McElhaney 1984; Williams 1998). For example, lipid composition may be regulated such that the membranes remain within the "lamellar fluid phase 'window' that extends from

Table 6: Summary of fatty acid composition (%) of skeletal muscle phospholipids and triacylglycerol of desert iguanas fed diets rich in saturated and unsaturated fats

Fatty Acid Species	Phospholipids		Triacylglycerol	
	Saturated Diet ($N = 3$)	Unsaturated Diet ($N = 4$)	Saturated Diet ($N = 3$)	Unsaturated Diet ($N = 4$)
15:0	6.88 (3.36)	5.92 (.96)	7.62 (2.80)	5.25 (4.43)
16:0	11.13 (4.99)	8.53 (2.31)	19.82 (5.55)	14.22 (3.07)
16:1	.32 (.56)	.00 (.00)	5.10 (8.83)	.00 (.00)
18:0	14.93 (4.75)	16.93 (3.17)	4.84 (3.01)	4.81 (1.48)
18:1n-9	12.08 (6.84)	7.93 (1.69)	15.85 (4.97)	13.45 (4.91)
18:2n-6	13.93 (3.94)	22.92 (3.89)*	7.63 (1.43)	33.98 (15.61)*
20:0	7.35 (10.10)	2.32 (.67)	5.40 (2.11)	3.02 (2.83)
20:1	2.67 (1.66)	4.87 (1.35)	7.05 (2.52)	6.62 (4.96)
20:2	.45 (.42)	1.04 (.14)*	1.34 (2.32)	1.18 (2.36)
20:3n-6	1.97 (1.72)	1.94 (1.30)	—	—
20:4n-6	14.23 (7.05)	9.80 (1.43)	.00 (.00)	.00 (.00)
20:5n-3	.88 (.21)	.95 (.13)	—	—
22:4n-6	.70 (.27)	.80 (.42)	—	—
22:5n-6	1.32 (.56)	2.13 (.90)	4.30 (1.90)	2.38 (2.35)
22:5n-3	2.13 (1.62)	1.96 (.28)	—	—
22:6n-3	9.06 (4.22)	11.97 (3.53)	21.07 (3.16)	15.10 (12.09)
UI	185.4 (33.5)	206.0 (17.4)	193.9 (27.0)	196.4 (46.2)
SFA	40.3 (.2)	33.7 (1.8)*	37.7 (4.9)	27.3 (3.2)*
UFA	59.7 (.2)	66.3 (1.8)*	62.3 (4.9)	72.7 (3.2)*
SFA/UFA	.7 (.0)	.5 (.0)*	.6 (.1)	.4 (.1)*
PUFA	44.7 (6.8)	53.5 (2.5)	34.3 (7.1)	52.6 (3.8)*
PUFA Diet	28.2 (3.7)	32.7 (5.1)	7.6 (1.4)	34.0 (15.6)*
PUFA >3 db	28.3 (9.3)	27.6 (3.2)	25.4 (5.0)	17.5 (14.4)
MUFA	15.1 (6.6)	12.8 (.8)	28.0 (8.3)	20.1 (1.0)
n6	32.1 (4.4)	37.6 (3.3)	4.3 (1.9)	2.4 (2.4)
n3	12.1 (2.7)	14.9 (3.4)	21.1 (3.2)	15.1 (12.1)
n6/n3	2.7 (.5)	2.7 (.9)	.2 (.1)	.1 (.1)

Note. Data are mean percentages (±SD). Abbreviations follow Table 2.

* Significant differences in tissue compositions between diet treatments at $P \leq 0.05$.

the thermotropic transition to the gel phase at low temperature to the formation of nonlamellar lipid phases at high temperatures" (Hazel et al. 1998, p. R861). Presumably, slight changes in fluidity, resulting from alterations in fatty acid composition, may not affect the phase of some portions of the membrane. This suggests that, ultimately, whole-animal responses may only be elicited if the phase of at least some discrete membrane domain is altered.

Composition of Tissues: Phospholipids and Triacylglycerol

Despite significant differences between treatments in the percentage of some individual fatty acids (see Tables 2–6), the expected significantly higher SFA/UFA ratios in the phospholipids of the saturated treatment group were only detected in adipose tissue and skeletal muscle and significantly lower in brain tissue (Fig. 1*B*). This result was unexpected because the SFA/UFA ratio of phospholipids is considered to correlate with membrane fluidity (Hazel and Williams 1990), with higher SFA/UFA ratios correlating with decreased fluidity and lower SFA/UFA ratios correlating with increased fluidity. Therefore, the lack of overwhelming differences between treatment groups in the SFA/UFA ratio in our study suggests that membrane fluidity of most tissues was also similar between the two treatment groups. An interesting discovery was the conservative nature of brain phospholipids between the treatments, despite the substantial differences in the dietary lipids. Indeed, among the tissues sampled, brain had the smallest differences between treatment groups. This suggests that preventing changes in brain fatty acids may be important to preserve the functionality of this organ. However, it is not clear by what mechanism(s) tissue composition is maintained.

Our results are in contrast to those from a similar study on lizards, which found that the total lipids extracted from tissues

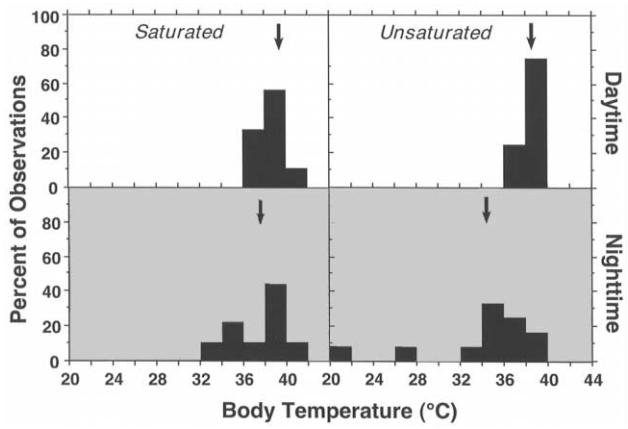

Figure 3. Mean body temperatures selected by lizards fed either diets rich in saturated or unsaturated fats during daytime and nighttime. Each observation is the mean body temperature for an individual calculated for the given time period (day or night). Arrows designate mean body temperatures for each treatment group.

corresponded directly with those in the diet (Geiser and Learmonth 1994). However, that study did not separate the lipids into classes and only examined extracts of total lipids. Hence, the differences in the saturation indices for the total-lipid extract reported by Geiser and Learmonth (1994) likely resulted from combining all lipids into a single class, including triacylglycerol, which is not known to affect membrane fluidity. Nevertheless, we cannot predict the outcome if all lipid classes (e.g., monoglycerides, cholesterol, etc.) were included in our study. Cholesterol, in particular, can play an important role in membrane fluidity by offsetting temperature-induced perturbations in membrane structure (Crockett and Hazel 1997; Crockett 1998). Future studies might consider isolating phospholipids from specific cellular membranes (e.g., plasma membrane, mitochondrial membrane, etc.); however, interpretation of such data might be complicated by the relatively cursory understanding of lipid metabolism (see Derickson 1976; Sheridan 1994) and what constitutes "normal" membrane-lipid composition in squamate reptiles (e.g., Morpurgo et al. 1993; Cartland-Shaw et al. 1998; Jones et al. 1998).

Selected Body Temperatures

When averaged across the two diet treatments, the mean T_{sel} of desert iguanas in the thermal gradient (daytime = 38.5° ± 1.2°C, $N = 21$; nighttime = 36.2° ± 4.1°C, $N = 22$; Fig. 3) were similar to those recorded for this species in other laboratory and field studies (e.g., Cowles and Bogert 1944; Cunningham 1966; McGinnis and Faulkenstein 1971; Pianka 1986). Body temperatures selected at night were significantly cooler for lizards fed the diet enriched with unsaturated fat than for lizards fed the saturated lipid diet. One potential explanation for this response is that, even at lower body temperatures, lizards fed the diet rich in unsaturated fats may still be able to assimilate the polyunsaturated dietary lipids or access their relatively unsaturated depot fat. This is because unsaturated fats remain in a fluid state even at low body temperatures (e.g., melting point of safflower oil ≈ −18°C). In contrast, the saturated treatment group may have been digestively "forced" to maintain higher body temperatures in order to assimilate the saturated lipids (e.g., melting point of coconut oil = 43°C) in their diet (Geiser et al. 1992). It is also possible that the different fatty acids in the diet elicit varying degrees of satiation such that an animal may alter its thermoregulatory behavior in response to how full it perceives its gut to be (see Regal 1966).

The fatty acid composition of brain tissues was highly conserved in our study (Table 3). Because the brain plays a role in thermoregulation in lizards (Cabanac et al. 1967), the lack of the expected changes in thermoregulation (i.e., lizards in the unsaturated treatment should select lower body temperatures than lizards in the saturated treatment) might be explained by the fact that the dietary lipids used in this study did not affect the phospholipid composition of brain tissues. As a result, the brain may not have produced differential, diet-induced thermoregulatory signals. However, it is important to note that other lipid constituents that were not measured (e.g., cholesterol) also affect membrane fluidity (Hazel and Williams 1990; Hazel 1995; Crockett and Hazel 1997; Crockett 1998; Williams 1998).

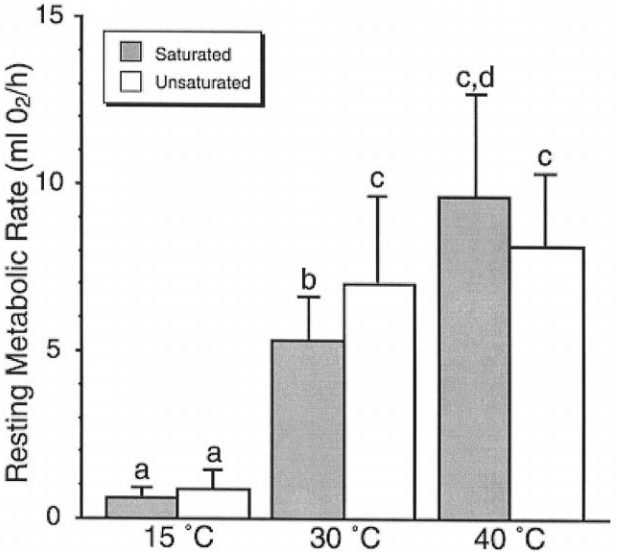

Figure 4. Resting metabolic rates (RMR) of the lizards on the unsaturated and saturated diet treatments at three ecologically relevant temperatures (mean + SD). Dissimilar lowercase letters indicate significant differences in RMR ($P < 0.05$). RMR is expressed unadjusted for body mass (see "Material and Methods").

Two of the lizards fed the diet enriched with unsaturated fats selected notably cooler nighttime body temperatures (Fig. 3). In nature, some desert lizards may exhibit individualistic patterns of nocturnal T_{sel} that are conserved over a season (Muchlinski et al. 1990), and a similar pattern of individual variation in T_{sel} was found for chuckwallas (*Sauromalus obesus*) in a thermal gradient (Nussear et al. 1998). Thus, it is possible that the low T_{sel} of these two individuals reflects individual preferences for particular nocturnal T_{sel} ranges rather than responses to the unsaturated diet or membrane fluidity.

The lack of profound differences in T_{sel} between treatments also might be explained by the fact that our saturated lipid diet contained some polyunsaturated lipids. However, Geiser et al. (1992) demonstrated a difference in T_{sel} among lizards fed an even lower proportion of dietary PUFAs (e.g., for the unsaturated diet, Geiser et al.: PUFAs = 32%, UI = 93.4, SFA/UFA = 0.3; this study: PUFAs = 62.4%, UI = 145.6, SFA/UFA = 0.2). The polyunsaturated lipids in the saturated diet likely originated from the Guinea pig chow. Nevertheless, our diets represent the extremes of major lipid constituents in natural diets consumed by herbivorous vertebrates (see Florant et al. 1990). Although a saturated diet could be formulated that lacks PUFAs, it would be difficult to distinguish responses resulting from differences in dietary fats from those resulting from the absence of required levels of some PUFAs (i.e., essential fatty acids; Staton et al. 1990). Moreover, such a diet is unlikely to be found in nature and, consequently, experiments of this type may not be ecologically relevant.

Critical Thermal Minimum

Our values of CTMin averaged over both diets were similar to those determined by other researchers for desert iguanas (Cowles and Bogert 1944). However, we did not detect significant differences in CTMin between the treatment groups. Animals with relatively more unsaturated membranes and/or depot fat were predicted to perform better at low body temperature because membranes should remain fluid and allow for the normal homeostatic processes that are mediated by cell membranes (e.g., propagation of action potentials). Although there were significant differences in the degree of saturation in the phospholipids of skeletal muscle, which should play a role in the righting reflex, we did not detect any difference in CTMin between the treatment groups. The absence of differences in the saturation index of brain phospholipids may explain this result because brain tissues, irrespective of diet treatment, would be expected to respond similarly to reductions in temperature. Alternatively, the fluidity or phase of the membranes at low body temperatures (ca. 14°C) may have been similar for both treatment groups and may have largely determined the responses of the lizards.

Nevertheless, the diet-induced changes in the fatty acid composition of depot fat may still be important, if animals are subjected to cold body temperatures for long periods of time (i.e., hibernation). The differences observed in triacylglycerol of adipose tissue suggest that lizards fed the unsaturated diet may be able to access these energy reserves at lower body temperatures, whereas lizards fed the saturated diet may not be able to access their depot fat. Temperature- and lipid-dependent accessibility of depot fat may be as important for overwinter survival in lizards as it is in small mammals (Florant et al. 1993; Frank 1994; Frank and Storey 1995). This hypothesis warrants further consideration and experimentation.

Resting Metabolic Rate

We found that the RMR of the unsaturated treatment group was significantly higher than the saturated treatment group when measured at 30°C; however, the treatment groups were not different when RMR was measured at either 15° or 40°C (Fig. 4). Additionally, we found that the RMR of the saturated treatment group nearly doubled between 30° and 40°C (a typical Q_{10} response; Bennett and Dawson 1976; Espinoza and Tracy 1997), whereas the RMR of the unsaturated treatment group did not significantly differ between 30° and 40°C. In theory, membranes that are more unsaturated may allow more ions to diffuse across the lipid bilayer (Hulbert and Else 1999, 2000). These ions must be actively pumped back across the membrane to maintain a transmembrane gradient. Animals with relatively more unsaturated membranes are predicted to have higher metabolic rates because of the additional energy expended to maintain the ion gradient (Hulbert and Else 1999, 2000). The absence of a difference in metabolic rate between treatment groups at 15° or 40°C in our study suggests that the unsaturated treatment group did not experience the predicted increase, relative to the saturated treatment group, in activity of its membrane-bound ion transporters at these temperatures. This may be explained by a lack of difference in the SFA/UFA ratio of the phospholipids between the two treatment groups for the metabolically most costly tissues (i.e., brain, heart, and liver). Overall, lizards fed diets rich in unsaturated and saturated lipids had temperature-specific RMRs that were within the range of those recorded for desert iguanas in other studies (see Andrews and Pough 1985).

The RMR of desert iguanas in the saturated treatment group nearly doubled between 30° and 40°C. In this case, perhaps some discrete membrane domains in the lizards fed the saturated diet were in a more ordered phase at 30°C and then transformed to a less ordered phase at 40°C. Phase transitions (from gel to fluid phase) of discrete membrane domains have been previously documented at normal physiological temperatures (Hazel et al. 1998). In contrast, the RMR of the unsaturated treatment group exhibited temperature independence in metabolic rate between 30° and 40°C. The lack of a detectable increase in metabolic rate from 30° to 40°C in the unsaturated treatment group, combined with the fact that the RMR of the

unsaturated treatment group was significantly higher than the saturated treatment group at 30°C, is provocative. It is possible that the unsaturated diet resulted in membranes that developed a higher degree of disorder (i.e., a phase transition) at a lower temperature than did the saturated treatment group. In this case, if the membranes of the unsaturated treatment group were similarly "leaky" (Hulbert and Else 1999, 2000) over this temperature range, we would predict that metabolic rate would be independent of temperature over this range. Other complex relationships between temperature and metabolic rate have been reported (Aleksiuk 1971; Bennett and Dawson 1976; Huey 1982), but this phenomenon has not been studied from such a mechanistic perspective. Our finding warrants a more detailed consideration of this phenomenon, with metabolic rate being measured at many more temperatures for lizards fed a range of experimental diets, or in other ectothermic taxa that may experience different dietary fats over latitudinal gradients (see Linder 2000). While almost all previous studies verify a general temperature dependence of metabolic rate across a wide temperature range, we expect that future empirical research at finer scales (e.g., metabolic rate measured at small intervals over a range of 10°C) may demonstrate temperature independence and lipid dependence of metabolic rate.

Finally, if the nighttime T_{sel} is lower in the unsaturated group, then the amount of energy required for metabolism in lizards fed the unsaturated diet would be lower than lizards fed the saturated diet (over the long term) because of the general positive relationship between metabolic rate and temperature in squamate reptiles (Andrews and Pough 1985).

Conclusions

Our study demonstrates that substantial differences in the composition of dietary fats alter the lipid composition of tissues and elicit concomitant differences in whole-animal physiological responses. Desert iguanas fed diets that differed in lipid content exhibited nighttime T_{sel}'s that were different between the treatment groups, RMR of the unsaturated treatment group was significantly higher than the saturated treatment group when measured at 30°C, and RMR increased significantly between 30° and 40°C in the saturated treatment group, whereas it exhibited temperature independence over this range in the unsaturated treatment group. No differences were found between treatment groups in selection of daytime body temperatures or CTMin. The absence of differences for some of the measured traits may be attributed to compensatory mechanisms preventing membrane fluidity or membrane phase from becoming functionally altered. Animals challenged by a diet low in PUFAs may synthesize longer-chained, more highly unsaturated lipids from existing, or rare, less complex PUFAs in order to maintain membrane fluidity. Likewise, lizards fed a diet rich in PUFAs may prevent their membranes from becoming too fluid or disordered by preventing the synthesis of long-chained, highly unsaturated lipids. From an ecological perspective, the diets we used represent the range of major lipid constituents in natural diets consumed by herbivorous vertebrates (data in Florant et al. 1990). Therefore, it would seem that under natural conditions, the lipids contained in a desert iguana's diet may affect ecologically important parameters like metabolic rate. Additional research is required to determine whether the significant differences found in the UI of triacylglycerol in adipose tissue is, in fact, advantageous to ectotherms in nature during prolonged periods at low body temperatures. The biochemical pathways involved in the synthesis and inhibition of membrane lipids are virtually unknown for reptiles, and further research in this area will offer insights into the mechanisms involved in homeoviscous adaptation and the potential importance of homeophasic adaptation to whole-animal biology among ectothermic vertebrates.

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