Temperature independence of the composition of triglyceride fatty acids synthesized de novo by the mosquito

EMILE VAN HANDEL

Florida State Board of Health, Entomological Research Center, Vero Beach, Florida

ABSTRACT The hypothesis that depot fat is more unsaturated when it is synthesized at lower temperatures was tested in the mosquito. Female mosquitoes (*Aedes sollicitans*) were starved until no triglycerides remained. A single dose of sugar was fed and the mosquitoes were maintained at different temperatures. Approximately the same amount of triglyceride was synthesized per mosquito at each temperature, although at different rates. Mosquitoes maintained at low temperatures did not synthesize more unsaturated triglycerides than those at higher temperatures: the fatty acid composition was essentially the same from 10 to 35°.

The triglycerides synthesized from sugar contained no polyunsaturated fatty acids. Total amounts and composition of phospholipid fatty acids remained unaltered during sugar feeding. When deprived of food, the mosquitoes catabolized triglyceride fatty acids randomly; cold-exposure did not cause selective retention or utilization of any individual fatty acid.

KEY WORDS temperature dependence · de novo synthesis · triglycerides · mosquito · adipose tissue · fatty acid composition · cold-exposure · poikilotherms · fatty acid oxidation

THE HYPOTHESIS of a correlation between environmental temperature and body fat composition dates back to 1901, when Henriques and Hansen reported that the dorsal subcutaneous fat of a pig exposed to cold had a lower melting point and a higher iodine value than that of a pig kept in a warm environment (1). This effect of cold-exposure, which has been confirmed by more recent observations, may be due to increased rates of synthesis of unsaturated fat, to increased rates of oxidation of saturated fat, or to a combination of these factors. The possibility that the difference in fatty acid composition is only an indirect effect of cold-exposure is suggested by Kodama and Pace, who found that in hamsters the same decreases in melting point and saturation of body fat that had been observed during cold-exposure occurred during semistarvation or during cortisone treatment (2).

An exception to the association of unsaturated fat and lower temperature is seen in the bat, an imperfect homeotherm. Unsaturation of triglyceride fatty acid in brown adipose tissue of this species is higher during the nonhibernating than during the hibernating season, when the body temperature is depressed (3). Poikilothermic animals are subjected to much greater internal temperature fluctuations and may therefore possess a more powerful mechanism to adjust body fat composition to temperature than do homeotherms. Fat of crustacean plankton is more unsaturated in polar seas than in moderate climates (4) and more unsaturated in winter than in summer (5). This may reflect a difference in the diet. When maintained at the same temperature, fat of fish feeding on winter plankton became more unsaturated than that of fish feeding on summer plankton (5).

Is adipose tissue fat more unsaturated when it is synthesized at lower temperatures? The introduction of mosquitoes for the study of triglyceride synthesis (6–8) provided an experimental approach to this question. The adult *Aedes sollicitans* can be starved until virtually no triglycerides remain and the triglycerides that appear after subsequent feeding on a fat-free diet are due to synthesis de novo. Only the female of this species synthesizes fat (6, 7). The present study describes the fatty acid composition of triglycerides synthesized from a single meal of sugar at temperatures between 10 and 35°.

TABLE 1 TIME COURSE OF FATTY ACID COMPOSITION OF TRIGLYCERIDES SYNTHESIZED BY FEMALE *Aedes sollicitans* AT 25°

Days After Feeding	Triglyceride Per Mosquito	Saturated			Unsaturated*		
		14:0	16:0	18:0	16:1	18:1	18:2
	mg	*% of total fatty acids*					
3.5 mg sugar per mosquito							
1	0.10	2.5	28	4.5	30	33.5	1.0
2	0.20	2.5	26	3.5	34	33	0.7
3	0.35	2.5	27	3.5	34.5	31.5	0.6
4	0.45	2.5	28	3.5	34.5	30	0.4
5	0.55	2.5	27.5	4.0	33.5	31	0.2
6	0.50	2.5	27	3.5	34.5	31	0.2
Sugar ad libitum							
12	0.60	3	29	3.5	33	31	tr.

* In addition, all samples contained 0.8–1% of 14:1.

METHODS

Biological Methods

Eggs obtained from *Aedes sollicitans* females brought from the field were stored for hatching at appropriate intervals. Larvae were reared (unless otherwise stated) at 27° on a liver–yeast–dog biscuit diet (9). Emerging females were isolated and maintained at 27° with only water available, with controlled 12-hr periods of light and darkness. After 7 days of starvation a single dose of sugar solution (equal parts of fructose and glucose) was fed by easing the proboscis of the mosquito inside a calibrated micropipette. In the time course experiment, 3.5 mg of sugar was fed; this was the maximum single dose the mosquito would tolerate. In the experiment to test temperature dependence, only 1.1 mg of sugar was fed because larger amounts were not well tolerated by mosquitoes maintained below 15°. All insects were fed on the same day and placed randomly in incubators set at 10 to 35°, with access to water only. Mosquitoes were removed at appropriate time intervals and analyzed in duplicate pools of at least 10 insects each. Each experiment was repeated three times.

Analytical Methods

The mosquitoes were extracted with chloroform–methanol 2:1, the methanol was removed with water, and the chloroform phase passed through a silicic acid column; a portion of the chloroform eluate was assayed for triglycerides (10).

The remainder of the chloroform eluate was saponified with alcoholic KOH, water was added, and the hexane-extractable material was discarded. The soaps were acidified with dilute sulfuric acid and the hexane-extractable fatty acids methylated with 3 N dry methanolic HCl at 65° for 30 min. Two volumes of chloroform were added and excess methanolic HCl was removed by repeated extraction with water. The chloroform was removed with a stream of nitrogen and the methyl esters were dissolved in hexane. Fatty acid composition was determined by gas–liquid chromatography, with ethylene glycol adipate polyester as the liquid phase and flame ionization detection.

When phospholipids were determined, the silicic acid columns were washed with a small amount of ether and the phospholipids eluted with methanol. After determination of lipid phosphorus (11), the fatty acids were analyzed as for triglycerides. As reported previously (6), the lipids in the chloroform eluate were almost exclusively triglycerides. Lipids determined by analysis of esterified glycerol (10) and by oxidation with bichromate (12) were not different.

In one experiment, the lipids of the chloroform eluates from the silicic acid columns were dissolved in hexane and rechromatographed on Florisil (13). The triglyceride fractions (eluted with 15% ether in hexane) had the same fatty acid composition as the chloroform eluates.

RESULTS AND CONCLUSIONS

Time Course Experiment

After 7 days starvation the mosquitoes contained 0–0.005 mg of triglycerides and 0.040–0.045 mg of phospholipids. When they were fed 3.5 mg of sugar and maintained at 25°, the phospholipid level did not change, but 0.55 mg of triglycerides was produced in 5 days. This represents utilization of 35% of the sugar carbon for triglyceride synthesis. The composition of the triglyceride fatty acids did not change, either during the period of rapid synthesis or during the period when the mosquitoes were fed on sugar ad libitum for 12 days (Table 1), except for the fivefold relative decrease in the percentage of linoleic acid. This acid is probably not synthesized from sugar and the small amount of nonphospholipid linoleic acid still present at the time of feeding is diluted by subsequent synthesis of other fatty acids. The conclusion, based on similar evidence, that mosquitoes do

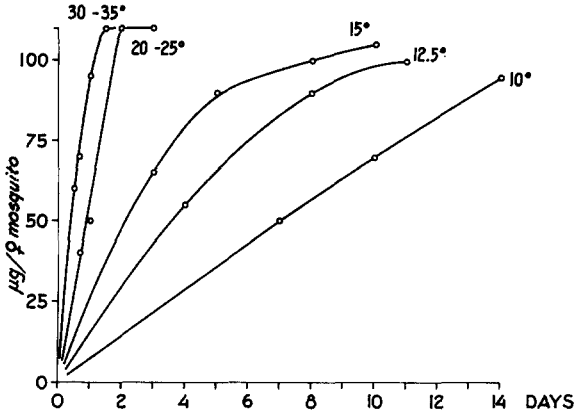

FIG. 1. Effect of temperature on the rate of triglyceride synthesis from a single dose of 1.1 mg of sugar fed to starved female *Aedes sollicitans*. The triglycerides present at zero time (0–5 µg per mosquito) have been subtracted.

not synthesize linoleic acid from sugar has been published previously (6).

Although the sugar-fed animals did not receive phosphorus, the phospholipid level did not fall below the initial 0.040–0.045 mg and the fatty acid composition remained the same. It included percentages of C_{16}–C_{18} acids similar to those in the triglyceride fatty acids, but the phospholipids contained in addition 15–18% of 18:2, 5–6% of 20:4 and 5–6% of 20:5. The latter two fatty acids were identified by carbon number (14) and emerged quantitatively as 20:0 (methyl arachidate) after hydrogenation.

Triglyceride Synthesis at Different Temperatures

The time course experiment established that the triglycerides have a constant composition both during rapid pool growth and during the period when the pool remained at its maximum (Table 1). One may therefore interpret a deviation from that composition as a direct effect of temperature on synthesis, when synthesis takes place at a different temperature.

The maximum amount of triglycerides synthesized by adult females from 1.1 mg of sugar was approximately the same from 10 to 35° (Fig. 1). At 30–35° the maximum was reached in 1 day, and at 10° in about 14 days. The fatty acid composition at the maximum, when 0.10–0.12 mg of triglycerides per mosquito had been synthesized, is presented in Table 2. The fatty acid composition at the time when only 0.05–0.06 mg had been produced was so similar to that in Table 2 that the data are not listed separately. Unsaturation was maximal, 65%, between 15 and 20°. At both higher and at lower temperatures it was only slightly less. These very small differences in a temperature interval of 25° indicate the absence of a temperature-dependent adaptive mechanism for fatty acid synthesis in the mosquito.

Triglyceride Utilization at Different Temperatures

When females were starved at 30, 20, and 10°, from emergence until 70–80% of the triglycerides had been catabolized, the fatty acid composition remained the same as at emergence (Table 3). This means that cold-exposure does not cause selective utilization or retention of any individual fatty acid.

Pupal Triglycerides at Different Temperatures

In the experiments in which the larvae were reared at 27° on a liver–yeast–dog biscuit diet, they pupated 5 days after hatching and emerged 1–2 days after pupation. Larvae reared at 30° pupated in 4 days, at 25° in 6 days, at 20° in 9 days, and at 15° in 18–20 days. At each of these rearing temperatures the pupae contained 0.16–0.18 mg of triglycerides and the fatty acid composition of the triglycerides was not different from that in Table 3.

DISCUSSION

Table 2 shows that the temperature at which triglycerides are synthesized from sugar by the adult female mosquito has no effect on triglyceride fatty acid composition. When starved adult females were fed ad libitum on sugar solution at 25°, the triglyceride pool reached a maximum of 0.55–0.65 mg in about a week and thereafter fluctuated only slightly. The composition of the triglyceride fatty acids during this "steady state" was not

TABLE 2 TRIGLYCERIDE FATTY ACIDS SYNTHESIZED FROM A SINGLE DOSE OF 1.1 MG OF SUGAR AT DIFFERENT TEMPERATURES BY FEMALE *Aedes sollicitans**

Temp.	Days after feeding	Saturated			Unsaturated†	
		14:0	16:0	18:0	16:1	18:1
°C		% of total fatty acids				
35	1	3	31	5	30	30
30	1	2.5	30	4.5	30.5	31.5
25	2	2.5	28	4	31.5	31
20	2	2.5	26	4.5	31.5	33.5
15	7	2	27	4	32	33
12.5	10	2	27.5	4	31	32.5
10	14	2.5	29	4	29.5	33

* Analyzed at the maximum triglyceride level (Fig. 1).
† All samples contained 0.5–1% each of 14:1 and 18:2.

TABLE 3 TRIGLYCERIDE FATTY ACIDS OF PUPAE AND OF NONFED ADULTS OF *Aedes sollicitans*

Saturated		Unsaturated	
% of total fatty acids			
14:0	2.5–3.5	14:1	1–2
16:0	26–29	16:1	20.5–23.5
18:0	5.5–7.5	18:1	26–29
		18:2	5–7
		20:4	1.5–2
		20:5	1.5–2

different from that during rapid growth of the fat pool (Table 1). This suggests that the fatty acids leaving the metabolic pool have the same composition as those entering the pool. Indeed, when these mosquitoes were starved until the triglyceride content had decreased from 0.60 to 0.10 mg, their fatty acid composition remained the same, which indicates that the fatty acids were oxidized randomly. Similarly, starvation from *emergence* until only 20% of the original triglycerides remained resulted in no change of fatty acid composition (Table 3).

The effect of environmental temperature on fatty acid synthesis de novo has been studied in microorganisms. Korn determined the total fatty acid composition in the soil ameba *Acanthamoeba* grown for 14 days on an artificial lipid-free medium at 15, 24, and 30°. It appeared that not only unsaturation, but also average chain length was somewhat greater at 15° than at 24° and 30°. It should be noted, however, that the amebas were sampled at different temperatures, but after the same time period; the lipid classes were not separated and the variations in fatty acid composition at a constant temperature were not investigated (15). Kates and Baxter studied the fatty acid compositon of the yeast *Candida lipolytica* at different incubation temperatures and found that the total fatty acids were richer in linoleic acid when the yeast was grown at 10° than when grown at 25°. The lipid classes were not separately analyzed. The fatty acid composition was not constant at a constant temperature; during the period of active growth the linoleic acid content varied greatly (16). These reports on the ameba and on the yeast cannot be interpreted as conclusive evidence for a direct effect of temperature on the composition of newly synthesized fatty acids.

In fed mosquitoes, the relative contribution of the polyunsaturated acids to the *total* fatty acid pool diminished with increasing temperature, since the triglyceride pool (poor in polyunsaturated acids) grows faster at higher temperature, whereas the level of phospholipids (rich in polyunsaturated acids) does not change. One day after receiving 1.1 mg of sugar, the mosquitoes contain 25% polyunsaturated acids at 10°, but only 6% at 30–35°. Therefore, data derived from analysis of *total* fatty acids cannot be used in resolving characteristics of de novo fatty acid synthesis at different temperatures. When starved mosquitoes are fed on sugar they synthesize about 0.1 mg of fat per day at 30°. Recently emerged mosquitoes maintained at 30° without food catabolize only about 0.025 mg of fat per day. It may be assumed that feeding greatly depresses this rate of fat utilization. The fat accumulating after feeding therefore reflects synthesis by adipose tissue primarily rather than an equilibrium between fat entering and fat leaving the pool.

When the insects are allowed to bite, and the precursor of the synthesized fat is predominantly blood protein, the fatty acid pattern is very similar to that derived from sugar. Apparently, the fatty acid composition of triglycerides synthesized by the female mosquito is independent of the precursor as well as of the temperature of synthesis.

The author acknowledges the technical assistance of Mr. J. W. Christ. This work was supported by a PHS Research Grant AI 05054 from the National Institutes of Health. The first analyses were performed in the Gaubius Institute of Leyden University, supported by a visitor's grant from the Dutch Organization Z.W.O.

Manuscript received 2 June 1965; accepted 23 August 1965.

References

1. Henriques, V., and C. Hansen. *Scand. Arch. Physiol.* **11:** 151, 1901.
2. Kodama, A. M., and N. Pace. *Federation Proc.*, **22:** 761, 1963.
3. Wells, H. J., M. Makita, W. W. Wells, and P. H. Krutzsch. *Biochim. Biophys. Acta* **98:** 269, 1965.
4. Lewis, R. W. *Comp. Biochem. Physiol.* **6:** 75, 1962.
5. Farkas, T., and S. Herodek. *J. Lipid Res.* **5:** 369, 1964.
6. Van Handel, E., and P. T. M. Lum. *Science*, **134:** 1979, 1961.
7. Van Handel, E. *J. Physiol.* **181:** 478, 1965.
8. Van Handel, E., and A. O. Lea. *Science* **149:** 298, 1965.
9. Lea, A. O. *J. Insect Physiol.* **9:** 793, 1963.
10. Van Handel, E. *Clin. Chem.* **7:** 249, 1961.
11. Bartlett, G. R. *J. Biol. Chem.* **234:** 466, 1959.
12. Pande, S. V., R. P. Khan, and T. A. Venkitasubramanian. *Anal. Biochem.* **6:** 415, 1963.
13. Carroll, K. K. *J. Lipid Res.* **2:** 135, 1961.
14. Woodford, F. P., and C. M. van Gent. *J. Lipid Res.* **1:** 188, 1960.
15. Korn, E. D. *J. Biol. Chem.* **238:** 3584, 1963.
16. Kates, M., and R. M. Baxter. *Can. J. Biochem. Physiol.* **40:** 1213, 1962.