# Distribution of radioactive glycerol and fatty acids among adipose tissue triglycerides after administration of glucose-U-$^{14}$C

C. H. HOLLENBERG

McGill University Medical Clinic, Montreal General Hospital, Montreal, Quebec, Canada

ABSTRACT Adipose lipid obtained from fed rats 15 or 60 min after injection of radioactive glucose was separated into 10 triglyceride classes of differing fatty acid compositions. The distribution among these classes of total and radioactive triglyceride-glycerol was determined and found to be the same. Thus newly synthesized adipose triglycerides resemble in kind and proportion the triglycerides which exist in the tissue. This finding is in accord with the concept that the structures of adipose triglycerides are stable over long periods and that the turnover rate of the several triglyceride species are similar.

After administration of radioactive glucose, the specific activity of saturated fatty acids was higher in the more saturated triglyceride species. These data indicate that newly formed saturated acids do not mix completely with all adipose tissue fatty acids available for esterification.

Fatty acids derived from plasma triglyceride influenced the composition of newly synthesized adipose tissue triglyceride and thus constitute an important source of adipose tissue lipid.

KEY WORDS rat · adipose tissue · triglycerides · argentation thin-layer chromatography · biosynthesis · fatty acids · immiscible pools

ALTHOUGH THE MANY DIFFERENT EXPERIMENTS that have been performed in vitro with adipose tissue have been invaluable in establishing the hormonal responsiveness and metabolic reactivity of this tissue, there is now some doubt as to whether these studies have provided an accurate picture of certain properties of this tissue when it is functioning under physiological conditions.

The exquisite reactivity of surviving adipose slices and particularly of free adipose cells to the lipolytic hormones, and the very considerable capacity of these preparations, when incubated with abundant glucose, to esterify free fatty acid suggest that stored adipose triglyceride is capable of rapid turnover, a process which would result in extensive intermolecular exchange of triglyceride fatty acids. However, in vivo experiments designed to study the rate of change of adipose triglycerides, indicate that these molecules are not subject to rapid alteration but are stable for many days (1–3). Similarly, although the remarkable capacity of incubated adipose slices and free cells for synthesizing fatty acids from glucose and acetate suggests that lipogenesis in situ may be the principal mechanism of lipid accretion in this tissue, recent in vivo studies have shown that only a small fraction of administered radioactive glucose can be recovered in white adipose tissue fatty acid (4–6). Other in vivo experiments suggest that in rats maintained on a mixed diet, plasma triglyceride may be a more important precursor of adipose tissue lipid than plasma glucose (3).

The present studies were designed to explore further the factors that regulate the formation and organization of adipose tissue triglycerides and to test some of the concepts suggested by in vitro experimentation. It was found that under physiological conditions, triglycerides formed in adipose tissue over a brief period resemble in kind and proportion the triglycerides that already existed in the tissue. Furthermore, acids derived from plasma triglyceride were found to occupy a significant fraction of the ester sites of $\alpha$-glycerophosphate that had been generated in adipose tissue during the experimental period. These results are in keeping with the concept that plasma triglyceride is a major precursor of adipose tissue lipid and that the structures of adipose triglycerides established at the time of triglyceride synthesis remain unchanged for

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

TABLE 1 FATTY ACID COMPOSITION (BY GLC) OF TRIGLYCERIDE CLASSES*

| Fatty Acids | Triglyceride Classes Derived from TLC | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | SSS | $SSU_1$ | $SU_1U_1$ | $SSU_2$ | $U_1U_1U_1$ | $SU_1U_2$ | $U_1U_1U_2$ | $SU_2U_2$ | $U_1U_2U_2$ | $U_2U_2U_2$ |
| | *mole %* | | | | | | | | | |
| S | 100 | 65 | 33 | 61 | 16 | 29 | 2 | 35 | 8 | 18 |
| $U_1$ | tr. | 35 | 67 | 10 | 75 | 35 | 65 | 4 | 34 | 27 |
| $U_2$ | 0 | 0 | 0 | 29 | 9 | 36 | 33 | 61 | 54 | 41 |
| $U_3$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | tr. | 4 | 14 |

S, 1 mole saturated; $U_1$, 1 mole monounsaturated; $U_2$, 1 mole diunsaturated.
* Lumbar fat was used in this study.

long periods thereafter. Other results suggest that adipose tissue contains several intracellular fatty acid pools that differ in fatty acid composition and that are simultaneously available to the esterifying system of the tissue.

## METHODS

Male Wistar rats (175–200 g) were used throughout the study; unless otherwise specified, the animals were maintained on Purina chow. In all experiments 20 $\mu$c of uniformly labeled glucose (Merck Sharp & Dohme, Montreal, Canada) was administered intravenously to lightly anesthesized animals, and 15–60 min later fat was removed from the lumbar, the epididymal, and occasionally the interscapular areas.

The adipose samples were extracted either in chloroform–methanol (7) or in isopropanol–heptane–water (8). Aliquots of the lipid extracts were used for GLC (9), measurement of radioactivity in saturated and monounsaturated fatty acids (3), and separation of major lipid classes by TLC (3). After preparative TLC, the total triglyceride fraction was eluted by repeated extraction of the adsorbent with diethyl ether, and the purified triglyceride was then separated into classes by a second application to thin-layer plates (20 × 40 cm) coated with silver nitrate-impregnated silica gel (10).

These plates were developed in benzene–diethyl ether 9:1 for 4 hr. After they had dried they were lightly sprayed with dichlorofluorescein and the various triglyceride fractions were located and marked under UV light. Ten well-delineated areas were consistently seen; lipid was eluted from these areas with diethyl ether and aliquots of the eluates were taken for counting and saponification (3). Recovery of radioactivity from the silver nitrate-impregnated plates always exceeded 90%. Aliquots of the fatty acids obtained after saponification were used for counting and for titration with 0.02 N NaOH. Radioactivity in triglyceride-glycerol in each triglyceride class was determined by the difference between the radioactivity in the whole triglyceride and that in the saponified fraction. The titration data were used for calculation of the distribution of total tissue triglyceride among the various triglyceride classes.

As Gordis has pointed out (2), preparative argentation TLC fractionates triglycerides not only according to degree of unsaturation but also to some extent on the basis of fatty acid composition. Table 1 indicates the GLC data obtained from a typical fractionation study. Triglyceride classes[1] SSS, $SSU_1$, and $SU_1U_1$ were obtained in practically pure form whereas the remaining classes were slightly contaminated with other fractions. In the procedure used, hexaunsaturated triglyceride remained at the origin; hence this fraction consistently contained not only trilinolein, the major component, but also more unsaturated triglycerides and free acids, and lower glycerides produced in small quantity during elution from the silica gel. The presence in the hexaunsaturated class of these breakdown products, which did not migrate from the origin, undoubtedly accounts for the saturated and monounsaturated fatty acids found in this fraction.

In some experiments, the specific activity of saturated acids synthesized in fat tissue from radioactive glucose was determined in certain triglyceride fractions. After the various triglyceride species had been eluted, the fatty acid methyl esters of each fraction were formed, separated by TLC, and eluted (3). The esters were hydrolyzed and aliquots of the saturated acid fractions were titrated and counted. Because many procedures were involved, only 75% of the fatty acid radioactivity initially isolated by preparative TLC was recovered in the final extracts. In these experiments all saturated fatty acids were treated as a group and possible differences between the metabolism of various saturated acids were neglected. This treatment was considered justified since palmitic acid comprised 75–80% of the total saturated acids of the tissue and, if one extrapolates from other data, probably a similar proportion of the radioactive saturated acids (11). It was recognized, however, that conclusions drawn from specific activity measurements could be misleading if there were

[1] S, $U_1$, $U_2$, represent saturated, monounsaturated, and diunsaturated fatty acids respectively. Triglyceride classes are represented by a triad of symbols designating the types of fatty acid present. No attempt was made to subdivide triglyceride classes on the basis of position of fatty acids or precise fatty acid composition. Hence $SSU_1$ designates all triglycerides containing 2 moles of saturated and 1 mole of monounsaturated fatty acid.

TABLE 2 SATURATED FATTY ACID COMPOSITION OF TRIGLYCERIDE CLASSES*

| Saturated Acid | Triglyceride Classes | | | | |
|---|---|---|---|---|---|
| | SSS | $SSU_1$ | $SU_1U_1$ $SSU_2$ | $SU_1U_2$ | $SU_2U_2$ |
| | | | *mole %* | | |
| Myristic | 7 | 8 | 6 | 8 | 6 |
| Palmitic | 79 | 81 | 80 | 81 | 77 |
| Stearic | 14 | 11 | 14 | 11 | 17 |

For explanation of symbols, see footnote 1 to text.

* Lumbar and epididymal fat were removed from three animals that had been fasted for 2 days and refed for a similar interval. The mean values of the three studies are shown.

significant differences between triglyceride classes in percentage composition of the saturated acid components. However, GLC analysis of five triglyceride classes revealed no marked differences (Table 2).

Plasma obtained at the time of excision of the adipose samples was extracted in the isopropanol–heptane–water system and plasma triglyceride was obtained by TLC. Aliquots of this fraction were used for GLC and for measurement of specific radioactivity of plasma triglyceride fatty acid.

The techniques used for assaying radioactivity have been previously described (3).

## RESULTS AND DISCUSSION

### *Distribution of Lipid Radioactivity among Adipose Tissues and Major Lipid Classes*

The data shown in Table 3 indicate that 15 min after the intravenous injection of labeled glycose, radioactivity was present in lipid extracts of all adipose depots studied; brown fat was the most heavily labeled and, of the white fat depots, lumbar tissue contained the most radioactivity on a wet weight basis. Lipid extracts of lumbar tissue contained about 0.1% of the injected radioactivity; 82% of this lipid radioactivity was present in triglyceride, and most of the remainder in diglyceride. At this time, the fraction of glyceride radioactivity present as glyceride fatty acid was variable; in seven studies of lumbar tissue, it averaged 12%, with a range of 2–49%. Variability in this proportion has been noted in other experiments in vivo (6, 12) and probably reflects variation between animals in the time of last feeding. At the 15 min interval, radioactivity was barely detectable in plasma triglyceride fatty acid.

### *Distribution of Total and Radioactive Glycerol among Triglyceride Classes*

Recent studies from several laboratories have indicated that in adipose tissue, transfer of fatty acids between triglyceride molecules occurs slowly (1–3). If these data accurately represent the in vivo behavior of total adipose tissue triglyceride, and if the fractional turnover rates of the many triglyceride species are similar, the triglycerides formed during a brief period of fat synthesis in adipose tissue should resemble, both in kind and proportion, the triglycerides already present in the tissue. The first group of experiments in the present study was designed to determine the validity of this concept.

Lumbar adipose tissue was removed from fed rats either 15 or 60 min after administration of radioactive glucose. The tissue triglycerides were isolated and then separated into 10 classes and the distribution among these classes of total and radioactive triglyceride glycerol was determined. Since the results at 15 and 60 min were identical, the data have been pooled to give Fig. 1.

The bulk of the tissue triglyceride was present in triglyceride classes containing one, two, or three double bonds; the $SU_1U_2$ class was the major component. The distribution of radioactive triglyceride glycerol among the 10 classes was nearly identical with that of total tissue triglyceride-glycerol. These data offer direct evidence that the definitive structure of adipose triglycerides is determined at the time of triglyceride formation and hence are entirely in keeping with the concept that these

TABLE 3 DISTRIBUTION OF LIPID RADIOACTIVITY IN DIFFERENT ADIPOSE TISSUES AND IN MAJOR LIPID CLASSES

| Adipose Tissue | Lipid Radioactivity | % Total Lipid Radioactivity | | | |
|---|---|---|---|---|---|
| | | TG* | DG | FFA | PL |
| | (*cpm* × $10^{-3}$/*g wet weight*) | | | | |
| Lumbar | 31.9 (24.3–36.2)† | 82(81–85) | 13(12–15) | 2 | 2 |
| Epididymal | 16.1 (14.9–18.2) | — | — | — | — |
| Mesenteric | 5.1 (4.5–6.0) | — | — | — | — |
| Interscapular | 186.0 | — | — | — | — |

Tissues were removed from three animals 15 min after administration of radioactive glucose and were extracted in chloroform–methanol. Interscapular fat was obtained from only one animal.

* TG, DG, FFA, PL represent triglyceride, diglyceride, free fatty acid, and phospholipid, respectively.

† Mean and range.

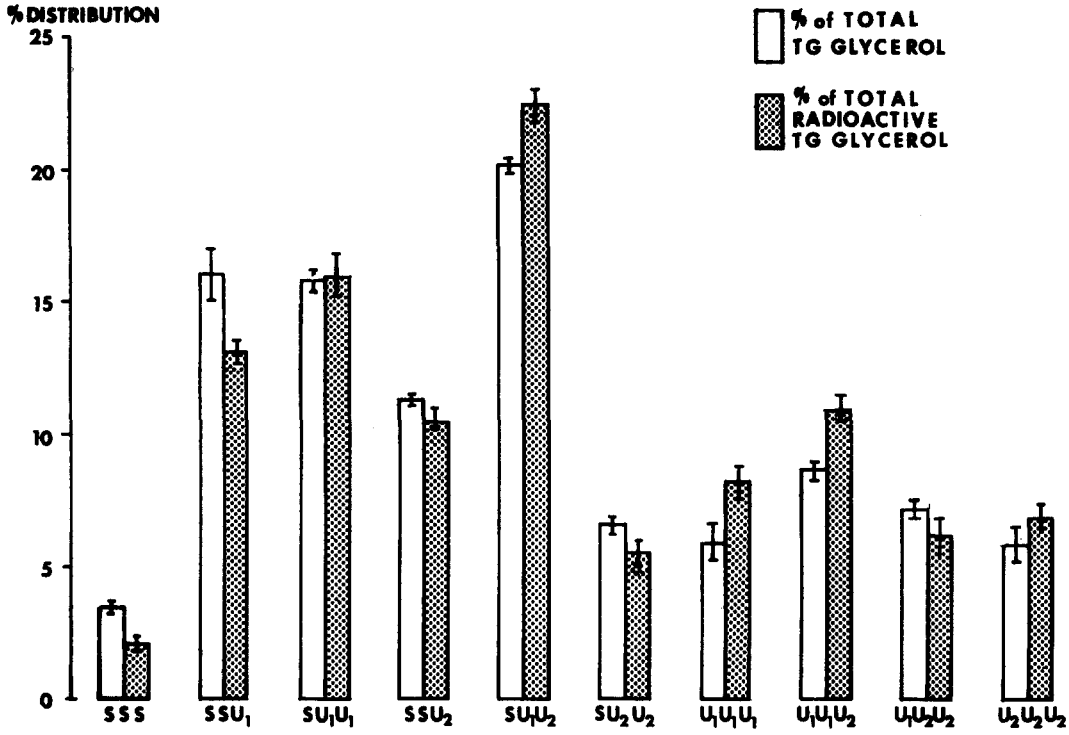


FIG. 1. Distribution of triglyceride glycerol among triglyceride classes. Mean values and SEM derived from six experiments are shown. In all instances lumbar fat was used; in four studies the tissue was removed 15 min after injection of radioactive glucose, and in two experiments this interval was 60 min. For explanation of symbols, see footnote 1 to text.

molecules are stable for long periods after synthesis. The data also imply a similarity in the fractional turnover rates of the triglycerides studied and indicate that the composition of the fatty acids that are esterified to newly formed $\alpha$-glycerophosphate closely resembles the composition of fatty acids in total tissue triglyceride.

### *Distribution of Newly Synthesized Fatty Acids among Triglyceride Classes*

In the experiments described the fatty acid components of the new triglycerides must have been derived from one or more of three sources: stored tissue lipid, plasma triglyceride, and acids synthesized during the course of the study. It was considered of interest to determine whether acids of all origins, available simultaneously to the esterifying system, were present in a homogeneous pool or in several pools of different fatty acid composition.

To explore this problem, we made use of the fact that after the administration of radioactive glucose, the newly formed adipose triglycerides resembled in kind and proportion the triglycerides existent in the tissue. These results imply that the distribution of saturated acids among the various species of new triglyceride must have been similar to the distribution among the same species of total tissue triglyceride. If newly synthesized radioactive saturated fatty acids had mixed completely with the entire fatty acid pool available for esterification, the specific activity of saturated acids in all triglyceride classes would have been identical. Alternatively, if the newly formed acids, composed of 80% saturated and 20% monounsaturated elements (3, 11), had been segregated entirely from acids of other origin, radioactive saturated acids would have been found only in classes SSS, $SSU_1$, and $SU_1U_1$, with most of this radioactivity being in the SSS and $SSU_1$ classes. Finally, if the new, predominantly saturated, radioactive acids had mixed with only a small part of the total free acid pool, the resultant fatty acid composition of this fraction would have been more saturated than that of the total quantity of acid simultaneously available for esterification. Thus although radioactive saturated fatty acids would have been recovered in all triglyceride classes containing saturated acids, the specific activity of these acids would have been greatest in the more saturated triglyceride species.

20 $\mu$c of uniformly labeled glucose was administered intraveneously to rats fasted for 2 days and then refed chow for a similar interval. Epididymal and lumbar fat were excised 15 min later and the samples were pooled and processed as previously described. Refed rats were used in order that the proportion of total lipid radioactivity in fatty acid would be enhanced; this proportion averaged 50% (range 17–70%). In conformity with previous observations (3), 80% of the fatty acid radioactivity

TABLE 4 DISTRIBUTION OF TRIGLYCERIDE GLYCEROL AMONG TRIGLYCERIDE CLASSES IN REFED ANIMALS

| Triglyceride Classes | Triglyceride Glycerol Distribution | |
|---|---|---|
| | Total | Radioactive |
| SSS | 3 | 3 |
| $SSU_1$ | 15 | 16 |
| $SU_1U_1$ } $SSU_2$ } | 26 | 28 |
| $U_1U_1U_1$ } $SU_1U_2$ } | 28 | 26 |
| $U_1U_1U_2$ } $SU_2U_2$ } | 16 | 13 |
| $U_1U_2U_2$ } $U_2U_2U_2$ } | 12 | 14 |

Data shown are the means of five experiments. SEM in all instances was 1 or less.

For explanation of symbols, see footnote 1 to text.

was recovered in saturated acids (range 68–87%); all of the remainder was in monounsaturated fatty acids.

The distribution of total and radioactive triglyceride-glycerol among the triglyceride classes studied is shown in Table 4. These results, obtained from refed animals, closely resemble those shown in Fig. 1 with respect to both the distribution of triglycerides among the classes and the identical distribution of preexistent and newly formed triglyceride glycerol. These results, together with the fact that only 50% of the total lipid radioactivity was present in the fatty acid fraction, indicate that fatty acids synthesized in the tissue over the experimental interval comprised only a small fraction of all acids esterified with α-glycerophosphate generated during this period. If acids synthesized de novo had formed a major portion of the fatty acid pool available for esterification, a much greater fraction of total lipid radioactivity would have been present as fatty acid and the new triglycerides formed would have been more saturated than those existent in the tissue.

Fig. 2 illustrates the relative specific activities of saturated acids in each of the five triglyceride classes containing saturated acids. In each experiment the specific activity of saturated acids in each class was expressed as a ratio of the specific activity of the entire quantity of saturated acid in all five classes.

Although radioactive saturated acids were recovered in all triglyceride classes studied, the specific activities of saturated acids in SSS and $SSU_1$ were higher than in those triglycerides containing three or four double bonds; saturated acids were present in highest specific activity in the $SSU_1$ class. It is evident that these results would not have been obtained had the newly synthesized acids either mixed completely with the total free acid pool or been completely segregated from acids of other origin. The results are in keeping with the concept that acids synthesized during the experimental interval had mixed with only a small portion of the fatty acid pool available for esterification and created a separate, relatively more saturated, precursor fraction. A similar conclusion was drawn from more indirect data obtained by incubation of adipose tissue in vivo with uniformly labeled glucose (3).

An alternative explanation of the data shown in Fig. 2 is apparent if it is assumed that acids synthesized during the course of the study were esterified predominantly to preexisting lower glycerides rather than to newly synthesized α-glycerophosphate, particularly if monoglycerides were to have been the predominant acceptors. This explanation still requires the postulation that newly formed acids were segregated from acids of other origin, unless it is assumed that the triglycerides formed from esterification of fatty acids of all origins with lower glycerides were more saturated than those formed from esterification of these acids with α-glycerophosphate. The quantitative importance of direct acylation of lower glyceride as a pathway of adipose triglyceride synthesis has not been established. Furthermore glucose, and hence α-glycerophosphate, does regulate fatty

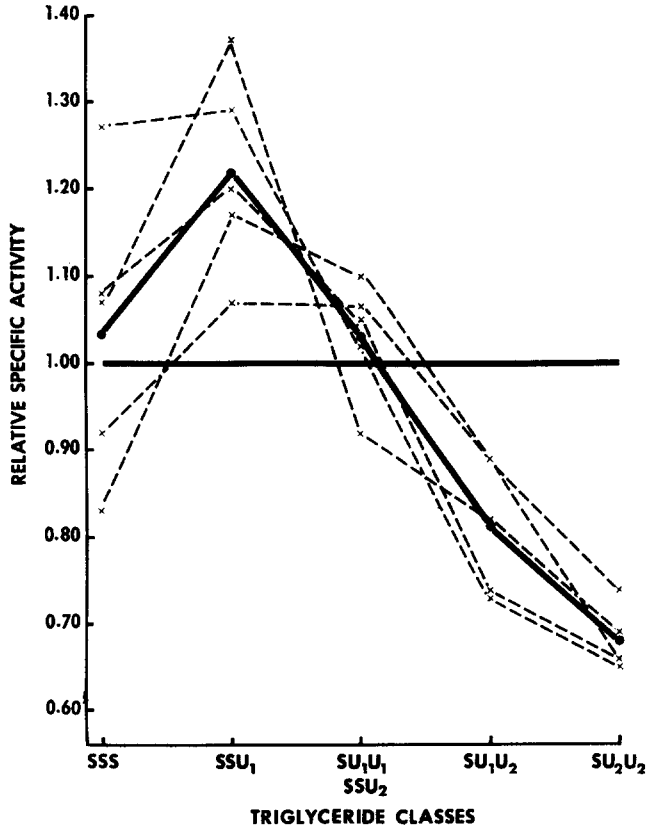


FIG. 2. Relative specific activity of saturated acids in various triglyceride classes. Data from five experiments are shown; mean values are indicated by the solid curve. In each study, the specific activity of saturated acids in each class was expressed as a ratio of the total specific activity of these acids in all five classes. For explanation of symbols, see footnote 1 to text.

acid esterification in this tissue and long-chain fatty acids are assimilated by fat tissue from refed rats principally—if not exclusively—by de novo synthesis of triglyceride (13). For these reasons it seems likely that in the present study the chief substrate for esterification of the newly formed acids was α-glycerophosphate.

Fatty acid esterification has been shown to be a function of the particulate fraction of the adipose cell (14, 15). The results of the present study suggest that acids synthesized in the tissue and those derived from stored lipid and plasma triglyceride are not mixed randomly on these particles, but are present in some order determined by their origin.

### *Influence of Fatty Acid Composition of Plasma and Adipose Triglyceride on Class Distribution of Radioactive Triglyceride Glycerol*

It has been mentioned previously that the fatty acid components of the new triglycerides must have been derived from stored tissue lipid, plasma triglyceride, and acids synthesized during the course of the experiments. It has also been shown that even under conditions of enhanced lipogenesis in adipose tissue, only a small fraction of all acids that were available to the newly formed α-glycerophosphate were synthesized during the experimental interval. Thus plasma or stored adipose lipid must have provided almost all of the acids so esterified. In vitro experiments have long suggested that most of the acids esterified with α-glycerophosphate in fat tissue originate in stored tissue lipid. The techniques of the present study were used to determine whether the contribution of acids derived from plasma lipid was at all comparable to the contribution from stored lipid. The fatty acid compositions of plasma and adipose triglycerides were made to be different from each other, radioactive glucose was administered, and the distribution of labeled glycerol among adipose triglycerides was determined. Under these circumstances, if the newly formed triglycerides were to have a different composition from those in the tissue, plasma triglyceride fatty acid would have contributed significantly to the fatty acid pool available for esterification.

The fatty acid composition of adipose triglyceride was established by feeding rats chow mixed with one of two fats, coconut oil or safflower oil, for a fairly long period; plasma composition was then changed in some animals by brief feeding of the other fat. At the conclusion of the feeding programs, 20 μc of radioactive glucose was administered and lumbar fat was removed 15 min later. In order to show more clearly any differences between the newly synthesized and existent triglyceride fractions, tri- and disaturated triglycerides were dealt with as a single group, as were those triglycerides containing 2 or 3 moles of diunsaturated fatty acid. This treatment was considered justified since manipulation of the content of saturated and dienoic acids would be expected to affect most profoundly the relative proportions of those triglycerides containing 2 or 3 moles of saturated or diunsaturated fatty acids. The results of these studies are shown in Table 5.

When chow, chow mixed with coconut oil, or chow mixed with safflower oil was fed, the fatty acid compositions of plasma and adipose triglyceride were similar (Expts. 1–3, 5). In these experiments the proportions of radioactive and total triglyceride glycerol in the saturated and diunsaturated triglyceride groups were similar. As expected, addition of coconut oil to the chow resulted in an increase in the proportion of the saturated

TABLE 5 Effect of Diet on Fatty Acid Composition of Plasma and Adipose Triglyceride and on Distribution of Adipose Triglyceride Glycerol

| Expt. | Feeding Regime* | Mole % Saturated Acids | | Mole % Diunsaturated Acids | | TG Glycerol Distribution | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Saturated† TG | | Diunsaturated‡ TG | |
| | | Plasma TG | Adipose TG | Plasma TG | Adipose TG | Radio-active | Total | Radio-active | Total |
| 1 | Chow | 34 | 37 | 29 | 24 | 25 | 31 | 20 | 20 |
| 2 | Coconut, 2 | 47 | 48 | 12 | 20 | 51 | 46 | 15 | 15 |
| 3 | Coconut, 5 | 51 | 57 | 17 | 14 | 57 | 66 | 10 | 8 |
| 4 | Coconut, 5; Safflower, 2 | 23 | 48 | 60 | 23 | 33 | 49 | 30 | 19 |
| 5 | Safflower, 5 | 28 | 25 | 59 | 45 | 15 | 18 | 41 | 39 |
| 6 | Safflower, 5; Coconut, 2 | 55 | 29 | 13 | 35 | 37 | 29 | 19 | 28 |
| 7 | Safflower, 7; Coconut, 2 | 51 | 34 | 21 | 41 | 35 | 29 | 23 | 33 |
| 8 | Safflower, 14; Coconut, 2 | 58 | 29 | 18 | 51 | 19 | 20 | 38 | 44 |

Lumbar fat was the source of all adipose samples. In the chow-fed animals, the adipose data were derived from six experiments, the plasma results from four of these six studies. In these studies fat was removed 15 or 60 min after administration of labeled glucose. In all other studies single animals were used and 15 min elapsed between injection and sampling.

* Fat was fed as 15% of the chow diet by weight for the number of days shown. In Expts. 4, 6, 7, 8 two diets were fed in the sequence indicated.

† Saturated triglycerides represent the SSS, $SSU_1$, and $SSU_2$ classes.

‡ Diunsaturated triglycerides represent the $SU_2U_2$, $U_1U_2U_2$, and $U_2U_2U_2$ classes.

triglycerides whereas feeding chow mixed with safflower oil raised the proportion of the diunsaturated triglyceride group.

In Expt. 4, as a result of the dietary regimen, the diunsaturated acid content of plasma triglyceride greatly exceeded that of adipose tissue. The proportion of radioactive triglyceride glycerol in diunsaturated triglycerides was considerably greater than the proportion of *total* triglyceride-glycerol in them, while this relationship was reversed in the saturated triglyceride group.

In Expts. 6 and 7 the diet sequence was reversed and the symmetrically opposite result to Expt. 4 was obtained. In Expt. 8, however, the proportions of radioactive and total triglyceride-glycerol in the saturated triglyceride group were identical although the expected difference between these proportions was evident in the diunsaturated classes. This variation between experiments undoubtedly reflects differences between animals in uptake of plasma triglyceride over the 15 min experimental interval. In Expt. 8, this uptake may have been influenced by the prolonged period of high fat feeding.

Thus, in most experiments, fatty acids derived from plasma triglyceride—or from an active adipose tissue compartment in equilibrium with this plasma lipid—had influenced the composition of triglycerides synthesized and must have contributed significantly to the fatty acid pool available to newly synthesized $\alpha$-glycerophosphate. Hence, under in vivo conditions, stored tissue lipid cannot be considered to be the only significant source of fatty acids available for esterification. These data provide direct evidence that plasma lipid is a major precursor of adipose tissue fatty acid and thus are in agreement with previous results which suggested that in growing, chow-fed rats, adipose lipid is derived predominantly from circulating triglyceride (3).

Mrs. Rosemary Mackay and Miss Maria Faluhelyi provided valuable technical assistance.

This work was supported by grant MT-872 from the Medical Research Council of Canada and by a grant from the Quebec Heart Foundation.

*Manuscript received 18 January 1967; accepted 8 March 1967.*

## References

1. Hollenberg, C. H. 1965. *J. Lipid Res.* **6:** 84.
2. Gordis, E. 1965. *J. Clin. Invest.* **44:** 1978.
3. Hollenberg, C. H. 1966. *J. Clin. Invest.* **45:** 205.
4. Patkin, J. K., and E. J. Masoro. 1964. *Can. J. Physiol. Pharmacol.* **42:** 101.
5. De Freitas, A. S. W., and F. Depocas. 1965. *Can. J. Biochem.* **43:** 437.
6. Himms-Hagen, J. 1965. *Can. J. Physiol. Pharmacol.* **43:** 379.
7. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. *J. Biol. Chem.* **226:** 497.
8. Dole, V. P. 1956. *J. Clin. Invest.* **35:** 150.
9. Hollenberg, C. H., and A. Angel. 1963. *Am. J. Physiol.* **205:** 909.
10. Barrett, C. B., M. S. J. Dallas, and F. B. Padley. 1963. *J. Am. Oil Chemists' Soc.* **40:** 580.
11. Benjamin, W., and A. Gellhorn. 1964. *J. Biol. Chem.* **239:** 64.
12. Goldman, J. K., and G. Cahill. 1964. *Metabolism.* **13:** 572.
13. Shapiro, B., M. Statter, and G. Rose. 1960. *Biochim. Biophys. Acta.* **44:** 373.
14. Goldman, P., and P. R. Vagelos. 1961. *J. Biol. Chem.* **236:** 2620.
15. Roncari, D. A. K., and C. H. Hollenberg. 1967. *Biochim. Biophys. Acta.* In press.