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TURNOVER OF ADIPOSE TISSUE TRIGLYCERIDES MEASURED BY THE RATES OF SYNTHESIS AND RELEASE OF TRIGLYCERIDE-GLYCEROL

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SUMMARY

In the epididymal fat pad from fed rats the amount of glycerol released due to endogenous lipolysis of triglycerides is balanced by synthesis of glycerol *de novo*. At a steady state, about 1.7 % of glyceride ester bonds are split and resynthesized per 24 h, which according to the first-order reaction rate renders an estimate of 41 days for the half-life of tissue triglyceride. The release of free fatty acids from the tissue is lower than that of glycerol, when compared on an equivalent basis. Measurement of fatty acid outflow relative to glycerol enables, therefore, to differentiate between the fatty acid retained within the tissue by re-esterification *in situ* and fatty acid exchanging with exogenous pools. The values for the "exogenous" turnover of fatty acid thus obtained represent only a fraction of the total turnover of ester bonds and fall into the range of similar data derived from fatty acid disappearance studies.

The ratio fatty acid outflow: glycerol release is increased on fasting and reduced on addition of glucose to the incubation medium or in tissues from rats refed after a period of fasting. In the latter, the surplus of glycerol synthesized over glycerol released is utilized for net deposition of external fatty acid. Glucose does not affect the amount of glycerol released, but reduces the fatty acid outflow by promoting glycerol synthesis. Lack of interdependence between esterification and lipolysis is thus indicated. In the presence of epinephrine, glycerol release grossly outbalances its synthesis, despite enhancement of the latter. Addition of large amounts of glucose suppresses the escape of fatty acid from such tissues, by raising the rate of esterification to that of lipolysis, and restoring the equilibrium at a higher level of triglyceride turnover.

INTRODUCTION

The steady state of adipose tissue is maintained through a dynamic equilibrium between esterification and hydrolysis of its TG. The FA freed by hydrolysis may, upon activation, be re-esterified with α-glycerophosphate, by a series of reactions outlined by ROSE AND SHAPIRO[1]. The glycerophosphate is most probably totally derived

Abbreviations: TG, triglycerides; FA, free (unesterified) fatty acids.

from the metabolism of glucose, since there is virtually no utilization of glycerol in adipose tissue[2], due to the lack of glycerokinase[3]. Any glycerol generated is therefore expected to diffuse out readily, as it appears to be metabolically inert in adipose tissue.

Based on these considerations an assessment of the turnover of adipose TG was undertaken by juxtaposing the synthesis of TG-glycerol as assayed by incorporation of glucose carbons, with the rate of TG-lysis as estimated by the loss of glycerol from the tissue. The values of glycerol outflow obtained, were then compared with those of FA esterification derived from measurement of the conversion of external medium FA into the tissue TG[4]. The release of tissue glycerol was also measured under conditions affecting TG esterification and lysis, and compared with the concomitant flow of FA. The data were then interpreted in terms of turnover of tissue TG and of its constituent moieties. A preliminary report on this subject appeared[5].

EXPERIMENTAL

Epididymal fat pads were excised from albino rats of Hebrew University strain, weighing 200–250 g. The tissues were rinsed in a 0.9 % NaCl solution and incubated in a 4 % solution of bovine albumin (Pentex, USA) at pH 7.4 in a shaking water bath at 37°. If not otherwise indicated, the incubation media in a final volume of 3 ml contained 3 mg glucose and about 4 μequiv of potassium palmitate. All measurements were made after an initial preincubation of 15 min, following which the tissues were rinsed and transferred into a fresh medium. [^{14}C]Glucose or [1-^{14}C]palmitic acid obtained from Amersham (Great Britain) were used. At the end of incubation the tissues were thoroughly rinsed in NaCl and albumin solutions, homogenized, extracted and processed as outlined previously[4]. An aliquot of medium was also extracted for FA determination. The heptane solution of tissue TG was divided into several aliquots. One was used for the determination of total counts incorporated into TG, while another aliquot of heptane was evaporated and hydrolysed by heating for 2 h at 60° with 0.5 N KOH solution in 75 % ethanol. After acidification, the FA were extracted with heptane and counted as described previously in a toluene scintillator[4]. An aliquot of the ethanolic phase containing glycerol was counted in BRAY's scintillator[6]. Correlation of the readings in the different scintillation media and correction for quenching was derived by the use of internal standards.

Glycerol was determined on an aliquot of medium, or in aqueous tissue homogenate, by a slightly modified method of BERGMEYER *et al.*[7], using glycerokinase and glycerophosphate dehydrogenase obtained from Boehringer (Germany). Deproteinization of the medium was carried out by the addition of equal volumes of matched $Ba(OH)_2$ and $ZnSO_4$ solutions. Total volume of the final reaction mixture was 1 ml including 0.1–0.3 ml of the sample. The final concentration of the reagents was as recommended by BERGMEYER *et al.*[7] except that half the amount of the glycine–hydrazine buffer (pH 9.8) was added.

RESULTS

Release of glycerol from adipose tissue

Release of glycerol *in vitro* from rat epididymal fat tissue was observed previously by LYNN *et al.*[8], LEBOEUF *et al.*[9], as well as in this laboratory[10], using the periodate

oxidation and chromotropic acid reaction for the assay of glycerol. Fig. 1 gives the time relationship of glycerol release in tissues from rats at various conditions, as determined with a specific enzymic method. It should be pointed out that in order to obtain a straight line corresponding to metabolic release, preincubation of the tissue was found necessary. The quantity of glycerol in epididymal fat pad, when ground immediately after excision from fed or fasted rats, ranged from 0.8 to 2.0 μmoles/g. An initial diffusion of part of the preformed tissue glycerol appears responsible for a disproportionate increment during the first minutes of incubation. The level of tissue glycerol drops during the preincubation and does not change appreciately on subsequent incubation. The release of glycerol into the medium continues linearly, even when large amounts are produced, as in the presence of epinephrine (Fig. 1).

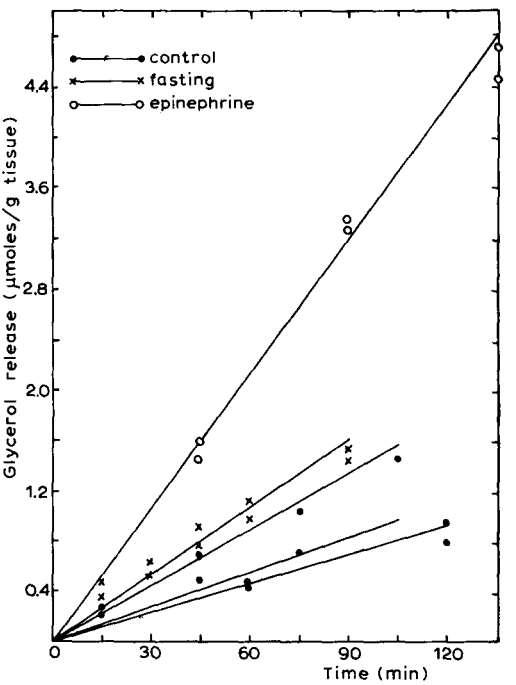

Fig. 1. Release of glycerol from epididymal fat pads *in vitro*. The tissues, weighing about 500 mg were preincubated for 15 min at 37° in 3 ml of a 4 % albumin solution containing 3 mg of glucose and the glycerol release at this time interval disregarded. In subsequent time intervals the glycerol increments were measured after the transfer of the tissues into a fresh medium of identical quantity and composition. Each line shows the results of a representative experiment. Epinephrine was present in the amount of 10 μg/g tissue.

Experiments were also carried out to exclude the possibility that part of the glycerol released may originate from the cleavage of tissue α-glycerophosphate, rather than from TG-lysis. The level of α-glycerophosphate as determined enzymically constitutes only a small fraction of that of preformed glycerol. On incubation of a fat-poor tissue homogenate fortified with α-glycerophosphate at pH 7.4, practically no increment in glycerol was encountered. This observation is similar to that of VAUGHAN[11] who found a weak α-glycerophosphatase activity in adipose tissue with

pH optimum between 5–6 and 9–10, well beyond the conditions applied throughout the present study.

Comparison of glycerol synthesis and release and FA esterification

Distribution of glucose carbons into adipose tissue constituents has been investigated by CAHILL *et al.*[12]. Table I records the results of similar measurements of glycerol and FA synthesis, as well as of glycerol and FA release and esterification performed on epididymal fat tissues from the same animal. Since the incorporation of glucose carbons into TG-glycerol has been found by CAHILL *et al.*[13] to follow a straight line with time, a comparison of simultaneous glycerol synthesis and release seems valid. As seen in Table I, a close agreement was found between amounts of glycerol synthesized and lost from tissues of fed rats incubated with [^{14}C]glucose. As only small changes in medium FA were observed, it may be inferred that most of the newly elaborated glycerol is utilized for re-esterification of FA arising from endogenous lipolysis of the TG and thus to replace the loss of TG-glycerol. Only a small fraction is required for the esterification of FA-synthesized *de novo* because the extent of lipogenesis from glucose is small compared with the rate of exchange of FA through lipolysis. The amount of palmitate esterified by the contralateral fat pad bathing in the [1-^{14}C]palmitate medium is somewhat less than equivalent to the amount of glycerol synthesized or released during the same time interval. Because TG formation requires equivalent amounts of glycerol and FA one should expect a close agreement between

TABLE I

TURNOVER OF GLYCEROL AND FATTY ACID MOIETIES OF ADIPOSE TISSUE TRIGLYCERIDES

Rat epididymal fat pads weighing 500 mg were incubated for 1 h in 3 ml of a 4 % albumin solution containing 3 mg of [^{14}C]glucose and about 4 μequiv of non-labeled potassium palmitate. Contralateral pads were incubated in a similar solution but with non-labeled glucose and [1-^{14}C]palmitate. Synthesis of FA *de novo* in μequiv was obtained by dividing counts/min incorporated into the fatty acid moiety of tissue TG by the specific activity of glucose carbons and dividing again by 17, their assumed average chain length. Glycerol synthesis in μmoles, was calculated by dividing counts/min found in TG-glycerol by the specific activity of glucose carbons and again by 3. The final value is expressed in thirds of μmoles in order to obtain direct comparison with μequiv of labeled palmitate esterified. The rate of esterification was measured as outlined in ref. 4. Release of glycerol was assayed enzymically on a sample of the medium.

Tissues from animals	*Fed*				*Fasted*			
Expt. No.	*I*	*II*	*III*	*IV*	*I*	*II*	*III*	*IV*
FA Synthesis μequiv/g/h	0.12	0.08	0.13	0.11	0.04	0.05	0.01	0.01
Glycerol synthesis 1/3 μmoles/g/h	2.72	2.17	1.98	1.86	3.18	3.81	4.12	4.44
Glycerol release 1/3 μmoles/g/h	2.13	2.40	1.72	1.92	6.90	7.14	6.15	5.55
FA esterification μequiv/g/h	1.60	1.80	1.42	1.23	1.37	1.15	1.40	1.04
Tissues from animals	*Refed*				*Fed + epinephrine*			
Expt. No.	*I*	*II*	*III*	*IV*	*I*	*II*	*III*	*IV*
FA Synthesis μequiv/g/h	0.33	0.21	0.39	0.19	0.01	0.01	0.00	0.00
Glycerol synthesis 1/3 μmoles/g/h	3.12	3.69	5.12	2.61	3.57	3.40	4.97	5.15
Glycerol release 1/3 μmoles/g/h	2.34	2.10	1.88	1.65	8.77	11.7	10.1	8.38
FA esterification μequiv/g/h	3.50	3.63	4.80	2.68	—	—	—	—

the synthesis of glycerol and incorporation of FA into TG. The possible reason for the observed difference may be due to a lag in equilibration between the radiopalmitate absorbed from the external medium and the FA arising from endogenous lipolysis. Thus, specific activity in the metabolic compartment at which re-esterification of FA occurs, is lower than that of medium FA. No such difficulty seems to exist in the availability of external glucose.

When tissues from rats fasted for 48 h are compared with those of fed rats, an increase in glycerol synthesis is evident but it is not sufficient to match the amount of glycerol lost, signifying an escape of TG-derived FA from the tissue. It should be remembered that the experiments of Table I were performed in the presence of glucose, which may in such tissues minimize the difference between the glycerol synthesis and release, by stepping up the former. In tissues obtained from rats refed for 48 h after a starvation period of 4 days, the synthesis of TG-glycerol is larger than glycerol appearing in the medium. The excess is required in part to esterify the newly synthesized FA, which rise to twice the amount found in formally fed rats. Esterification of FA absorbed from the medium is also enhanced, and in contradistinction to the tissues from fed rats quantitatively exceeds that of glycerol release. This finding indicates that most of the glycerol surplus is utilized for the net conversion of medium FA to TG.

When epinephrine, 10 μg/g tissue, is included in the incubation medium, the synthesis of TG-glycerol increased to levels comparable to those of fasting rats, but is surpassed by the release of glycerol. The large discrepancy between the glycerol lost and replaced accounts for the marked release of FA, well known to occur on stimulation with epinephrine. FA esterification cannot be accurately measured in this condition because the tissue deviates from the steady state, the external FA rise marked during incubation and incorporation of FA into TG being retarded with time[14].

Correlation of glycerol release and FA outflow

In Table II the rate of FA outflow from the tissue is compared on an equivalent basis with the release of glycerol. The FA outflow includes net release of FA by the tissue, as well as that portion of TG-derived FA which exchanged with medium FA. As seen in Table II tissues from fed rats incubated in absence of glucose remain practically in a steady state as far as medium FA uptake and release is concerned. The ratio of FA outflow to glycerol indicates that about 70 % of endogenously lysed FA are re-esterified *in situ*. Since there is little change in medium FA it may be concluded that the remaining 30 % of TG-derived FA are exchanged with those of medium. Addition of glucose to the medium with the contralateral fat pad does not significantly affect the amount of glycerol contributed to the medium. The increased elaboration of glycerol in such tissue enables net uptake of medium FA and promotes endogenous re-esterification. The ratio of FA outflow: glycerol indicates that as much as 85 % of FA produced by TG lysis are retained within the tissue.

Like in tissues of fed rats, the release of glycerol from rats starved for 48 h does not seem to be affected by the addition of glucose *in vitro*. The release and outflow of FA, however, are markedly diminished in the presence of glucose. As indicated by the FA outflow: glycerol ratio, inclusion of glucose elevates the esterification capacity of the tissue from 25 to 60 % of the FA originating from within. Thus the state of tissues from fed rats incubated without glucose is approached.

TABLE II

RELATION OF GLYCEROL RELEASE TO FA OUTFLOW FROM ADIPOSE TISSUE

Incubation of epididymal fat pads was carried out as outlined in Table I except that glucose was omitted in controls. The outflow of the FA from the tissue was obtained by dividing the counts per min of [1-^{14}C]palmitate remaining in the medium at the end of incubation by its initial specific activity (counts/min/μequiv) and subtracting the value obtained from the FA content of the medium at the end of incubation. In experiments with epinephrine 9 mg of glucose and 5 μg of the hormone were present in the medium with 500 mg tissue instead of 3 mg of glucose in other experiments. Standard deviation $\pm$ SD was calculated from the formula $\sqrt{\Sigma(d)^2/(n-1)}$ where d denotes deviation from the mean and n the number of experiments.

		Fed		*Fasted*		*Refed*		*Fed + Epinephrine*	
	Tissues:	*with*	*without*	*with*	*without*	*with*	*without*	*with*	*without*
		Glucose		*Glucose*		*Glucose*		*Glucose*	
Glycerol release	Mean	2.23	1.92	4.92	4.74	2.19	1.88	8.21	8.00
1/3 μmoles/g/h	S.D.	$\pm$0.51	$\pm$0.46	$\pm$1.64	$\pm$0.95	$\pm$0.31	$\pm$0.63	$\pm$1.91	$\pm$2.29
FA release*	Mean	—0.48	0.09	0.75	2.92	−1.19	−0.82	1.20	4.85
μequiv/g/h	S.D.	$\pm$0.79	$\pm$0.07	$\pm$0.55	$\pm$1.35	$\pm$0.38	$\pm$0.26	$\pm$0.93	$\pm$1.45
FA outflow	Mean	0.36	0.56	2.02	3.27	0.10	0.19	1.99	5.35
μequiv/g/h	S.D.	$\pm$0.25	$\pm$0.17	$\pm$0.63	$\pm$1.33	$\pm$0.05	$\pm$0.08	$\pm$1.32	$\pm$1.51
FA outflow	Mean	0.15	0.29	0.40	0.64	0.04	0.13	0.22	0.69
Glycerol release	S.D.	$\pm$0.08	$\pm$0.09	$\pm$0.14	$\pm$0.20	$\pm$0.016	$\pm$0.10	$\pm$0.11	$\pm$0.11
No. of experiments:		10	10	10	10	7	10	8	10

* Negative sign indicates uptake of FA from the medium.

Tissues from refed rats avidly esterify FA from medium while their FA outflow is correspondingly low. The rate of endogenous lipolysis does not differ much from that of fed rats, and is only slightly influenced by glucose *in vitro*. The resulting ratio FA outflow: glycerol signifies the re-esterification of close to 90 % of FA arising from TG lysis. In the presence of glucose there is practically a complete retention of tissue FA.

Addition of epinephrine to a medium with tissues of fed rats elevates the tissue glycerol output about 4-fold, irrespective of the presence of glucose. The FA increment likewise reaches high values, but may be effectively suppressed by glucose. Thus, while glucose does not influence the rate of epinephrine-enhanced lipolysis, it enables, through the augmented glycerol synthesis, the containment of most of the FA freed within the tissue. This is exemplified by an average increase in the re-esterification from 30 to 70 % of the cleaved ester bonds, equivalent to the glycerol released. Thus in the wake of a greatly increased breakdown of glucose in epinephrine-stimulated tissue, the equilibrium between esterification and lipolysis tends to be restored. The small loss of FA in the presence of glucose occurs now at a much higher level of TG turnover and represents only a small fraction of FA originating from TG lysis.

DISCUSSION

A balance between glycerol synthesis and glycerol release has been demonstrated in adipose tissue excised from fed rats. In such tissue close to equilibrium with respect

to FA in- and outflow, the amount of glycerol surrendered by lipolysis or re-synthesized from glucose actually represents the turnover of TG, provided that lipolysis of a triglyceride molecule, once begun, proceeds until the liberation of free glycerol. If products of partial lipolysis are re-esterified to any significant extent, the values obtained apply only to the turnover of ester bonds of a complete molecule. The turnover of the FA moiety in such case would exceed that of glycerol, commensurate with the rate of esterification of di- or monoglycerides.

WADSTROM[15] reported the appearance of lower glycerides in rabbit subcutaneous fat tissue after administration of epinephrine. He found an increase in lower glycerides from about 0.3 to 3% of the total tissue glyceride content. Such accumulation of intermediaries during enhanced lipolysis does not necessarily imply that they are utilizable as substrates for esterification. Indeed, the ratio resulting from the division of FA outflow (in μequiv) by glycerol release (in thirds of μmoles) does not exceed unity (Table II). Conservation of TG-glycerol through re-esterification of lower glycerides would require a higher ratio. Furthermore, if re-utilization of products of incomplete lipolysis is prominent, measurement of esterification by incorporation of medium radiopalmitate into TG should render higher values than glycerol synthesis. The opposite result is obtained in Table II for tissues of fed rats. It is also pertinent that diglyceride has been excluded as a significant FA acceptor in rat adipose tissue by the experiments of SHAPIRO *et al.*[16].

Mobilization of FA from adipose tissue may be effected either by TG-lysis as evident from the release of both FA and glycerol, and/or by decrease in endogenous reesterification, as seen from the increase of the FA: glycerol ratio in the receiving medium. In fasting or in the presence of epinephrine (Table II) a comparison of the rate of FA outflow with that of glycerol release reveals that the former is pronounced beyond the accounted increase in lipolysis. Reduction in re-esterification is due then to the insufficient glycerol synthesis. In fasting, inavailability of glucose makes the reason obvious, while under epinephrine, diversion of glucose into catabolic pathways seems responsible for the relatively inadequate elaboration of TG-glycerol[14]. Upon inclusion of glucose in the incubation media, the esterification of FA is sufficiently promoted to restrain FA outflow, but there is little effect on the rate of lipolysis indicating no interrelation between these two processes. The dissociation of esterification from lipolysis is further demonstrated in Table III, where rise in esterification caused by the increase in medium FA concentration did not provoke a change in glycerol release. Glycerol synthesis, however, has been shown by CAHILL *et al.*[13] to rise in response to the increased demand for esterification induced by high levels of medium FA. The rate of lipolysis remains also unaffected when esterification in epididymal fat pads *in vitro* is suppressed in the presence of KCN, CH_2ICOOH or NaF (see ref. 17), indicating that the decrease in esterification does not in itself activate lipolysis of tissue TG.

While these findings point to the significance of α-glycerophosphate as the acceptor of FA for re-esterification and as the determinant of TG turnover in rat tissues both *in vitro* and *in vivo*[18], this may not be the case in other species. In humans, glucose administration reduces the output of glycerol by adipose tissue, indicating conservation of tissue glycerol by promoting re-esterification of partially cleaved glycerides[18].

The value of 0.7 μmole of glycerol split and resynthesized/g tissue/h seems

TABLE III

FA ESTERIFICATION AND GLYCEROL RELEASE AT DIFFERENT MEDIUM FA CONCENTRATIONS

Epididymal fat pads weighing about 500 mg were excised from rats fed *ad libitum* and given 10% glucose solution instead of drinking water for 24 h before the experiment. The tissues were incubated for one hour in 3 ml of a 4% albumin solution containing [1-^{14}C] palmitate. Glucose was omitted from the medium. The two tissues from one rat were incubated each at different FA concentration as indicated. The rate of esterification was calculated in μequiv g/h as in ref. 4. Glycerol release is given in thirds of μmoles/g/h.

	Initial medium FA μequiv							
	1.45		*2.87*		*4.14*		*9.09*	
	FA esterification	*Glycerol release*	*FA esterification*	*Glycerol release*	*FA esterification*	*Glycerol release*	*FA esterification*	*Glycerol release*
Expt. 1	0.73	1.65	1.31	1.60				
Expt. 2	0.98	2.60	1.86	2.50				
Expt. 3					2.86	2.35	5.80	2.35
Expt. 4					2.54	1.23	5.43	1.33

representative of the TG turnover in epididymal tissues of fed rats. Disregarding partial lipolysis, this would mean that about 17 μmoles of TG are completely exchanged/24 h in epididymal fat pads of a 200-g rat, which usually weigh 1 g. The TG content of such tissue amounts to about 90% of its wet weight, which corresponds to about 1000 μmoles of TG, taking 900 as the average molecular weight. About 1.7% of tissue TG molecules undergo then a complete exchange daily. Calculation of half-life of tissue TG according to the first-order reaction rate renders an estimate of 41 days. This should be considered as an average value for the whole tissue since esterification and lipolysis in adipose tissue has been shown to proceed at different rates in various compartments of the tissue[4].

It seems of interest to compare the half-life of TG as derived from the above considerations with the half-life obtained from disappearance of labeled FA from fat depots. The tissues TG have been labeled by the authors either by feeding rats with different FA[19], feeding ^3H_2O (see ref. 20), or by direct exposure of epididymal fat pads to FA by the technique of STEIN AND STEIN[21]. The values obtained in these studies after a long term follow up range from 60 to 190 days. It should be remembered that FA disappearance rate pertains to exchange of FA originating in the tissue with other body pools and does not encompass the FA which are freed and re-esterified *in situ*, with newly synthesized glycerol. Contrariwise, the presently determined glycerol turnover time of 41 days corresponds to the sum of endogenous and "exogenous" tissue FA turnover. The "exogenous" turnover may be estimated by using the FA outflow: glycerol ratios of Table II. In tissues from fed rats in the absence of glucose on the average 29% of the FA equivalent to glycerol escapes re-esterification, indicating that the exogenous half-life of FA equals that of glycerol multiplied by

the factor of 100/29. The resulting figure of 141 days compares well with those obtained from FA disappearance. In the presence of glucose it increases to 273 days. After 48-h fast with fat content lowered to 80 % of tissue wet weight and glycerol release of 1.6 μmoles/g/h, the TG-half-life shortens to about 16 days and the "exogenous" half-life of FA to 25 days. On refeeding, the half-life of TG returns to normal but the "exogenous" half-life of tissue FA lengthens to several hundred days. It appears then, that determination *in vitro* of the ratio of FA outflow to glycerol release may serve as a convenient estimate of total and "exogenous" tissue TG turnover at various physiologic conditions.

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