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The substrate determines the rate and pattern of neutral lipid synthesized by isolated human sebaceous glands

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Lipogenesis from different substrates was determined in isolated human sebaceous glands after 17–20 h in culture. Rates of total lipogenesis were 1003 ± 141, 842 ± 90, 481 ± 57 pmol·h^{-1}·gland^{-1} ±SE from acetate, lactate and glucose, respectively, when present as sole substrates: the rate from glucose was significantly lower ($P < 0.01$). Squalene synthesis was greatest from acetate at 479 ± 44 pmol·h^{-1}·gland^{-1}; significantly higher than from lactate (281 ± 45 pmol·h^{-1}·gland^{-1}) or glucose at 119 ± 18 pmol·h^{-1}·gland^{-1}. Wax ester plus cholesterol ester synthesis showed similar dependence on substrate but triglyceride synthesis was unaffected. We conclude that the added substrate determines both the rate and pattern of non-polar lipid synthesized by isolated human sebaceous glands.

Sebaceous lipid; Squalene; Triglyceride; Wax ester; Cholesterol ester; (Sebaceous gland)

1. INTRODUCTION

The neutral lipids found in sebum are characterised by unusually large content of wax ester and squalene [1], neither of which are major products of lipogenesis in other lipid synthesizing tissues.

In vivo studies using subcutaneous injection of labelled substrates suggested preferential utilisation of acetate over glucose for wax ester and squalene synthesis [2]. This conflicted with conclusions drawn from experiments with whole skin [3].

Until recently, study of lipid synthesis by sebaceous glands could only be inferred from freshly collected sebum [4] or from incorporation of labelled precursors into whole skin lipids with subsequent dissection of the glands for analysis [5]. The procedure of Kealey et al. [6], now enables the isolation of viable sebaceous glands from small pieces of human skin. We report the use of this technique to show that human sebaceous glands carry out extremely rapid lipogenesis from a variety of precursors in vitro and that the pattern of non-polar lipid produced depends on the nature of the precursor.

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2. MATERIALS AND METHODS

[1-^{14}C]acetate, [U-^{14}C]glucose, [U-^{14}C]lactate, L-[U-^{14}C]isoleucine and [^{3}H]cholesteryl oleate were from Amersham International. [^{3}H]Squalene was from Dupont (England). Pure lipids were from Sigma (England).

Sebaceous glands were obtained by shearing [6] from samples of facial or neck skin removed during routine dermatological out-patient operations. Glands of approximately equal size were usually cultured for 17–20 h in Dulbecco's MEM (Flow Laboratories) containing 10% (v/v) fetal bovine serum (Imperial Laboratories), penicillin and streptomycin. Lipogenesis by pairs of glands was measured in 0.2 ml of Krebs bicarbonate buffer with unlabelled substrate plus 0.37 MBq of ^{14}C-labelled substrate in 5% CO_2 + 95% O_2. Rates were maximal by 4 mM for each substrate but, following the approach of Anderson and Dietschy [7], substrates were presented at concentrations reflecting their potential to form acetyl-CoA, (1 mol acetyl-CoA/mol acetate, 2 mol acetyl-CoA/mol glucose, etc.). Thus 8 mM acetate and lactate were used for comparison with 4 mM glucose and isoleucine. After 6 h at 37°C the reaction was stopped by freezing. Both total and individual lipid synthesis had been shown to be linear for at least 6 h. Lipids were extracted

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Table 1

Rate of incorporation of [1-^{14}C]acetate into total lipid (dpm · h^{-1} · gland^{-1} ± SE)

Substrate	Freshly isolated glands	Precultured glands
Acetate, 8 mM ($n=4$)	993 ± 237	3350 ± 345*
Glucose, 8 mM ($n=4$)	7977 ± 3785	24106 ± 4872*

* $P<0.05$ with respect to rate with freshly isolated glands

from medium plus glands [8] after addition of a ^{3}H recovery standard. The washed, $CHCl_3$ layer was divided; part was counted to give the total lipid synthesis, part was separated into individual non-polar lipids by thin-layer chromatography [9]. Before thin-layer chromatography, mass standards of pure lipids and human sebaceous lipid were added, together with ^{3}H recovery standards. Individual lipids were identified by staining with I_2 vapour and cut out for radioactivity determination.

3. RESULTS AND DISCUSSION

Overnight culture of glands with DMEM plus fetal bovine serum stimulated lipogenesis 3-fold ($P<0.05$, table 1). With 8 mM [^{14}C]acetate alone the rate rose from 361 ± 149 to 1218 ± 217 pmol · h^{-1}gland^{-1} ± SD. Incorporation into all major lipid classes was similarly increased. All subsequent experiments were carried out on precultured glands.

Our results were obtained under defined conditions in vitro using labelled substrates at 4 or 8 mM as sole carbon sources. This was to obtain an estimate of absolute rate of lipogenesis without significant dilution of the cellular acetyl-CoA pool by endogenously derived unlabelled material. Under these conditions, the human sebaceous gland carried out rapid and sustained lipogenesis from acetate, lactate and glucose (table 2) although the rate of incorporation of glucose into total lipid was about half ($P<0.01$) the value obtained with acetate and lactate. It is clear that there is no special requirement for added glucose for sebaceous lipogenesis, contrary to suggestions [10].

Table 3

Substrate	^{14}C tracer	Ratio of synthesis rates squalene/triglyceride (mean ± SD)
Acetate, 8 mM ($n=14$)	[1-^{14}C]acetate	2.0 ± 0.7*
Lactate, 8 mM ($n=22$)	[U-^{14}C]lactate	1.0 ± 0.5*
Glucose, 4 mM ($n=12$)	[U-^{14}C]glucose	0.5 ± 0.2
Glucose, 8 mM ($n=15$)	[1-^{14}C]acetate	0.5 ± 0.2
Isoleucine, 4 mM ($n=14$)	[U-^{14}C]isoleucine	0.4 ± 0.2

*$P<0.01$ with respect to [^{14}C]glucose incorporation

L-Isoleucine, a possible precursor for the methylated fatty acids found in sebaceous lipid [11], was also a substrate for total lipogenesis but at only 20% of the glucose rate. Since labelled squalene was formed from [^{14}C]isoleucine, the amino acid was clearly being catabolised to acetyl-CoA (and propionyl-CoA). The latter could then form both odd-numbered and methylated fatty acids, as suggested by Nicolaides [12].

With all the substrates used in this study, the non-polar lipids made up 80–90% of the total lipid synthesized. The remainder was phospholipid (not shown). The pattern and rate of [^{14}C]glucose incorporation into non-polar lipids closely followed those reported by Cassidy et al. [13] with this

Table 2

Rates of lipid synthesis from various ^{14}C precursors (pmol · h^{-1} · gland^{-1} ± SE

Lipid class	[^{14}C]Acetate (8 mM) ($n=14$)	[^{14}C]Lactate (8 mM) ($n=22$)	[^{14}C]Glucose (4 mM) ($n=12$)	[^{14}C]Isoleucine (4 mM) ($n=14$)
Total lipid	1003 ± 141***	842 ± 90***	481 ± 57	102 ± 32***
Squalene	479 ± 44***	281 ± 45**	119 ± 21	22 ± 4**
Wax ester plus cholesterol ester	76 ± 8*	75 ± 8*	44 ± 7	24 ± 7
Triglyceride	256 ± 27	339 ± 48	261 ± 31	54 ± 11**

*$P<0.05$, **$P<0.02$, ***$P<0.01$ with respect to glucose incorporation

substrate. However, both the absolute rate (table 2) and relative rate (table 3) of squalene synthesis were significantly greater when lactate or acetate replaced glucose as sole substrate. This was not simply due to increased availability of acetyl-CoA since: (i) rates of triglyceride synthesis were similar from glucose, acetate and lactate (table 2), and (ii) squalene synthesis was not significantly increased at 8 mM glucose (143 ± 60 vs 119 ± 73 $pmol \cdot h^{-1} \cdot gland^{-1}$ ± SD). The clearest demonstration of this 'directing' effect of different substrates is shown in table 3. In this table the data are presented as the ratio of the rate of squalene synthesis to the rate of triglyceride synthesis for each substrate. Here tracer [^{14}C]acetate label followed the glucose labelling pattern (squalene: triglyceride rate ratio = 0.5 ± 0.2) in the presence of 8 mM glucose. This was significantly ($P<0.01$) less than the ratio of 2.0 ± 0.7 obtained from the same tracer in the presence of 8 mM acetate. Our observations explain the report [14] that, in incubations of whole skin in glucose containing medium, [^{14}C]acetate gave the same pattern of non-polar lipid synthesis as [^{14}C]glucose. This shows the difficulties inherent in the interpretation of mixed substrate experiments.

The explanation for the apparent 'directing' effect of substrate may be that de novo synthesis of the fatty acids comprising a molecule of triglyceride requires 4 times as much NADPH as does the synthesis of a molecule of squalene. Metabolism of acetate, unlike glucose and lactate, does not produce NADPH which would have to be provided from endogenous sources when acetate was a precursor. This limitation could encourage divertion of cytoplasmic acetyl-CoA to squalene synthesis when supplied at high rates from acetate.

Our results are also relevant to the observations [4,15] that prolonged starvation in man caused a 2-fold increase in the ratio of squalene to triglyceride concentration in sebum. This change might reflect alterations in the substrates available to the sebaceous gland under these conditions. On prolonged starvation glucose availability decreases relative to concentrations of blood lactate and ketone bodies and even acetate [16]. Thus, in vivo, the sebaceous gland may use a variety of substrates for lipogenesis. While acetate may never be of major importance (maximum blood concentration 1 mM) it is significant that skin is extremely active at conversion of glucose to lactate [17] resulting in high local concentrations of lactate for sebaceous lipogenesis.

REFERENCES

[1] Downing, D.T. and Strauss, J.S. (1974) J. Invest. Dermatol. 66, 228–244.
[2] Colton, S.W. and Downing, D.T. (1985) Biochim. Biophys. Acta 837, 190–196.
[3] Wheatley, V.R., Hodgkins, L.T., Coon, W.M., Kumasariri, M., Berenzweig, H. and Feinstein, J.M. (1971) J. Lipid Res. 12, 347–360.
[4] Pochi, P.E., Downing, D.T. and Strauss, J.S. (1970) J. Invest. Dermatol. 55, 303–309.
[5] Summerley, R. and Woodbury, S. (1971) Br. J. Dermatol. 85, 424–431.
[6] Kealey, T., Lee, C.M., Thody, A.J. and Coaker, T. (1986) Br. J. Dermatol. 114, 181–188.
[7] Andersen, J.M. and Dietschy, J.M. (1979) J. Lipid Res. 20, 740–752.
[8] Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911–917.
[9] Cooper, M.F., McGrath, H. and Schuster, S. (1976) Br. J. Dermatol. 94, 369–373.
[10] Hsia, S.L., Dreize, M.A. and Marquez, M.C. (1966) J. Invest. Dermatol. 36, 237–239.
[11] Wheatley, V.R., Chow, D.C. and Keenan, P.D. (1961) J. Invest. Dermatol. 36, 237–239.
[12] Nicolaides, N. (1971) Lipids 6, 901–905.
[13] Cassidy, D.M., Lee, C.M., Laker, M.F. and Kealey, T. (1986) FEBS Lett. 200, 173–176.
[14] Summerley, R., Yardley, H.J., Raymond, M,, Tabiowo, A. and Ilderton, E. (1976) Br. J. Dermatol. 94, 45–53.
[15] Downing, D.T., Strauss, J.S. and Pochi, P.E. (1972) Am. J. Clin. Nutr. 25, 365–367.
[16] Newsholme, E.A. and Leach, A.R. (1983) Biochemistry for the Medical Sciences, Wiley, Chichester, New York.
[17] Freinkel, R.K. (1960) J. Invest. Dermatol. 34, 37–42.