Br. J. Pharmac. (1983), **80**, 663–670

Age related changes in the antilipolytic effects of nicotinic acid in rat adipose tissue

L. Caparrotta, G. Fassina, R.M. Gaion & F. Tessari

Institute of Pharmacology, University of Padova, Largo E. Meneghetti 2–I 35100 Padova, Italy

1 The effect of nicotinic acid on lipolysis was tested *in vitro* in adipose tissue from three groups of rats, selected according to age: 6–7 weeks, 10–12 weeks and 16–20 weeks old.

2 Although the changes were not statistically significant, the basal release of free fatty acid (FFA) was increased and glycerol was decreased by nicotinic acid (0.01–1 mM); the drug caused a statistically significant increase in basal FFA: glycerol ratio in a concentration-dependent manner. This ratio also increased with age in the absence of drug.

3 (−)-Noradrenaline (10 μM) and theophylline (3 mM) each stimulated lipolysis. When glycerol release was calculated as a percentage increase, the effects of these drugs became more marked with age. By contrast, the highest absolute rate of induced release occurred in adipose tissue from the youngest rats.

4 The lipolytic effect of 10 μM (−)-noradrenaline was generally unaffected by nicotinic acid except in adipose tissue from the oldest rats when the glycerol release was reduced by 1 mM nicotinic acid, although it did not alter FFA: glycerol ratio.

5 The stimulation of glycerol release induced by 3 mM theophylline was not affected by the presence of nicotinic acid in the youngest rats, but the drug elicited a concentration-dependent antilipolytic effect in adipose tissue from 10–12 weeks old rats, which was even more pronounced in the oldest animals. Lower theophylline concentrations (0.6–1 mM) were also sensitive to nicotinic acid inhibition in the 6–7 weeks old rats. In the presence of theophylline, nicotinic acid had no effect on FFA: glycerol ratio.

6 These data show a direct influence of age on the antilipolytic action of nicotinic acid.

Introduction

In a theoretical consideration of the treatment of hyperlipidaemias as related to the mechanism of action of various lipid lowering drugs, our attention was focused on three simple observations which might be correlated: (a) a high percentage of human hyperlipidaemias have a late onset in life (Bierman & Ross, 1975; Carlson, 1975; Dioguardi & Vergani, 1978) and aging by itself causes a progressive increase of plasma lipids within limits that are considered non-pathological (Walton, 1974; Rifkin, La Rosa & Heiss, 1980); (b) in adipose tissues, which is the major lipid store in the body, many metabolic functions undergo age-related changes (Gellhorn & Benjamin, 1965; Forn, Schönhöfer, Skidmore & Krishna, 1970; Reardon, Goldrick & Fidge, 1973; Holm, Jacobsson, Björntorp & Smith, 1975); (c) some of these functions are also affected by nicotinic acid (Krishna, Weiss, Davies & Hynie, 1966; Shafrir, Orevi & Gutman, 1971; Skidmore, Schönhöfer & Kritchevsky, 1971).

These observations led us to wonder if a correlation might exist between age and the effect of nicotinic acid. The fact that altered metabolic conditions, such as starvation and alloxan diabetes, have been shown to influence the effects of this drug in adipose tissue and other organs (Solyom & Puglisi, 1966; Otway, Robinson, Rogers & Wing, 1971; Shafrir *et al.*, 1971) gives further support to such conjecture. In fact, aging is associated with a variety of metabolic alterations, which may be related to the occurrence of disease processes (Goldstein & Harley, 1969; Masoro, Bertrand, Liepa & Yu, 1979; Roth, 1979). Aging may thus be considered as an altered metabolic condition and, as such, it would potentially affect nicotinic acid action.

In order to test this hypothesis the effect of nicotinic acid was studied in rats of various ages. Since inhibition of lipolysis in adipose tissue is considered as a primary mechanism of the drug's action (Carlson, 1963; Hepp, Dietze & Wieland, 1971; Carl-

son, 1978), this system was chosen for the present *in vitro* experiments. Free fatty acid (FFA) and glycerol release were measured, both basally and after (−)-noradrenaline or theophylline stimulation.

The present results show that the antilipolytic action of nicotinic acid increases with age, suggesting a possible specific relationship between the pharmacological properties of this drug and metabolic alterations caused by aging.

Methods

Three groups of male Wistar rats were used, selected according to age: 6 to 7 weeks old (180 ± 10 g body weight); 10 to 12 weeks old (350 ± 20 g body weight); 16 to 20 weeks old (500 ± 20 g body weight).

Epididymal fat pads were excised under light ether anaesthesia, washed in saline, rapidly cut into 1–2 mm large strips and randomized.

Aliquots of 100 ± 5 mg of minced tissue were suspended in 1.9 ml of Krebs Ringer bicarbonate buffered solution (pH 7.4) containing bovine serum albumin fraction V, 2.5% unless otherwise indicated. The composition of the Krebs Ringer solution was (mM): NaCl 109, KCl 4.5, $CaCl_2$ 2.7, KH_2PO_4 1.2, $MgSO_2$ 1.2, $NaHCO_3$ 24.2.

Nicotinic acid (dissolved in 50 μl ethanol) was added to the incubation medium where indicated. The same volume of ethanol was added to the samples in which the drug was not present. The samples were then preincubated for 30 min at 37°C in a metabolic shaker before the addition of the lipolytic drugs. (−)-Noradrenaline or theophylline, dissolved in 50 μl of 0.9% w/v NaCl solution (saline), was then added to the samples which were further incubated for 150 min. Thus nicotinic acid was present in the samples throughout the preincubation period until the end of the incubation (180 min total). The reaction was stopped by adding 0.1 ml of 2.5 N H_2SO_4. Aliquots of 1.0 and 0.3 ml of the medium were then used for the determination of FFA and glycerol respectively.

FFA were titrated according to Dole (1956) and glycerol was determined according to the methods of Lambert & Neish (1950) by the use of acetylacetone reagent proposed by Nash (1953). Nicotinic acid did not interfere with these determinations at any of the concentrations used. Student's paired *t* test was used in statistical analyses and *P* values of less than 0.05 were considered statistically significant.

Bovine serum albumin fraction V was purchased from Sigma, St. Louis, Missouri. (−)-Noradrenaline bitartrate monohydrate was from Recordati, Milano; theophylline from Carlo Erba, Milano; nicotinic acid from Merck, Darmstadt. All the other chemicals were of analytical grade.

Table 1 Effect of age and nicotinic acid on basal lipolysis in rat adipose tissue

Drugs (mM)	*FFA* (μEq g^{-1} fresh tissue, 180 min^{-1})	*Glycerol* (μmol g^{-1} fresh tissue, 180 min^{-1})	*FFA: glycerol*
	6–7 weeks		
None	6.18 ± 0.92	3.08 ± 0.55	2.01 ± 0.32
NA 0.01	6.52 ± 0.77	2.35 ± 0.38	2.77 ± 0.39
NA 0.1	7.06 ± 0.75	2.19 ± 0.27	3.22 ± 0.38*
NA 1	7.10 ± 0.26	2.26 ± 0.28	3.14 ± 0.41*
	10–12 weeks		
None	4.06 ± 0.89*	1.15 ± 0.18***	3.53 ± 0.43
NA 0.01	4.59 ± 0.77	1.03 ± 0.33	4.46 ± 0.62
NA 0.1	5.60 ± 1.02	0.89 ± 0.15	6.29 ± 0.60*
NA 1	5.31 ± 0.75	0.76 ± 0.13	6.97 ± 0.72**
	16–20 weeks		
None	4.01 ± 0.31**	0.95 ± 0.10***	4.22 ± 0.50
NA 0.01	4.59 ± 0.76	0.87 ± 0.05	5.28 ± 0.61*
NA 0.1	4.91 ± 0.54	0.82 ± 0.10	5.99 ± 0.63**
NA 1	5.08 ± 0.57	0.81 ± 0.07	6.27 ± 0.58**

Epididymal fat pads were isolated from three groups of rats: 6–7 weeks, 10–12 weeks and 16–20 weeks old. Albumin concentration in the incubation medium was 2.5%. NA = nicotinic acid. Each value represents the mean ± s.e.mean of 9 to 12 assays from 5 different experiments. FFA: glycerol ratio was calculated from the values obtained in the single experiments. Statistical significance of the difference from the control in young rats (first line of each column) is indicated as: *$P < 0.025$; **$P < 0.01$; ***$P < 0.001$.

Table 2 Effect of nicotinic acid on noradrenaline-induced glycerol release as related to age

Drugs (mM)	*Glycerol* (μmol g^{-1} fresh tissue, 150 min^{-1})	*FFA: glycerol*
	6–7 weeks	
None	2.91 ± 0.24	1.95
Noradrenaline 0.01	17.84 ± 0.55	2.14
Noradrenaline 0.01 + NA 0.01	18.09 ± 1.15	2.10
Noradrenaline 0.01 + NA 0.1	19.60 ± 0.35	1.82
Noradrenaline 0.01 + NA 1	19.77 ± 1.05	1.84
	10–12 weeks	
None	1.14 ± 0.81	2.00
Noradrenaline 0.01	10.30 ± 0.81	2.00
Noradrenaline 0.01 + NA 0.01	—	—
Noradrenaline 0.01 + NA 0.1	8.65 ± 1.24	2.53
Noradrenaline 0.01 + NA 1	8.24 ± 1.20	2.45
	16–20 weeks	
None	0.97 ± 0.11	4.12
Noradrenaline 0.01	10.93 ± 1.04	1.84
Noradrenaline 0.01 + NA 0.01	11.61 ± 0.79	1.82
Noradrenaline 0.01 + NA 0.1	8.74 ± 1.09	2.03
Noradrenaline 0.01 + NA 1	7.55 ± 0.69*	2.31

Epididymal fat pads from rats 6–7 weeks, 10–12 weeks and 16–20 weeks old were used for incubation in Krebs Ringer solution (pH 7.4) containing 2.5% albumin. NA = nicotinic acid. Each value represents the mean ± s.e.mean from 4 different experiments. Statistical significance of the difference from noradrenaline 10^{-5} M; *$P < 0.02$.

Results

Basal lipolysis

The spontaneous release of FFA and glycerol from adipose tissue obtained from rats 6–7 weeks, 10–12 weeks and 16–20 weeks old is reported in Table 1. FFA: glycerol ratios, calculated from the values obtained in each experiment, are also included.

In 10–12 weeks and 16–20 weeks old animals the rates of both FFA and glycerol release (expressed per g fresh tissue) were significantly lower than those of the youngest rats. The FFA to glycerol ratio, which is independent of the reference unit (g of tissue), increased with age.

The changes induced by nicotinic acid (0.01 to 1 mM) on basal lipolysis are not statistically significant (Table 1). However, in all the age groups the drug caused a small increase in the release of FFA while reducing the release of glycerol. As a result of these changes the FFA: glycerol ratio was substantially enhanced by nicotinic acid in a dose-dependent manner.

Noradrenaline-induced lipolysis

In adipose tissue from all animals, lipolysis was highly stimulated by 10 μM (−)-noradrenaline (Table 2). Under our experimental conditions this effect in the two groups of younger rats was not altered by nicotinic acid, at any of the concentrations tested (0.01 to 1.0 mM). Only in the oldest rats the highest concentration of nicotinic acid (1 mM) significantly antagonized the lipolytic action of (−)-noradrenaline. Glycerol is shown as an index of lipolysis and parallel results were obtained on FFA (data not shown). In these conditions, i.e. in the presence of (−)-noradrenaline, FFA: glycerol ratio

Table 3 Effect of nicotinic acid on noradrenaline-induced lipolysis with different concentration of albumin

Drugs	*FFA* (μEq g^{-1} fresh tissue, .150 min^{-1})	*Glycerol* (μmol g^{-1} fresh tissue, .150 min^{-1})	*FFA: glycerol*
	2.5% albumin		
None	3.53±0.45	2.17±0.71	1.63
Noradrenaline 1 μM	20.58±2.11	9.78±0.32	2.10
Noradrenaline 1 μM + NA 1 mM	18.25±2.45	8.95±0.98	2.04
Noradrenaline 2 μM	27.21±2.71	12.45±0.99	2.19
Noradrenaline 2 μM + NA 1 mM	28.51±2.54	12.94±1.01	2.20
	5% albumin		
None	5.73±1.47	2.49±0.24	2.30
Noradrenaline 10 μM	39.66±3.56	17.74±0.68	2.24
Noradrenaline 10 μM + NA0.1 mM	36.92±4.21	18.65±0.83	1.98
Noradrenaline 10 μM + NA 1 mM	35.42±2.69	19.90±1.06	1.87

Epididymal fat pads were from 6–7 weeks old rats. Albumin medium concentration was 2.5% and 5% in the first and second group of experiments respectively. NA = nicotinic acid. Each value represents the mean ± s.e.mean of the data obtained from 6 incubations in 3 experiments.

was not affected either by age or by nicotinic acid (Table 2).

In order to discover if this lack of nicotinic acid effect in younger animals might be due to the fact that the concentration of (−)-noradrenaline used was supramaximal, or if the albumin added to the medium had reached saturation by FFA, thus causing a feed-back inhibition of lipolysis, further experiments were performed altering the concentrations of noradrenaline and albumin. As shown in Table 3, nicotinic acid did not exert any antilipolytic effect either in the presence of submaximal (−)-noradrenaline concentrations (1 and 2 μM), or when the albumin concentration was doubled to 5%.

Theophylline-induced lipolysis

In adipose tissue from the youngest rats, 3 mM theophylline caused a 7 fold increase in the rate of lipolysis (Table 4). Neither FFA nor glycerol release induced by theophylline was significantly altered by the addition of nicotinic acid. In 10–12 weeks old rats the addition of 3 mM theophylline resulted in an 8 fold increase of lipolysis, even though the absolute rate of the process (expressed as μmol of glycerol g^{-1} fresh tissue, 150 min^{-1}) was 36% lower than in the youngest animals (Table 4). In rats of the same age group (10–12 weeks) nicotinic acid elicited a concentration-dependent inhibition of theophylline-stimulated glycerol release which was reduced by 42% by the highest concentration of nicotinic acid.

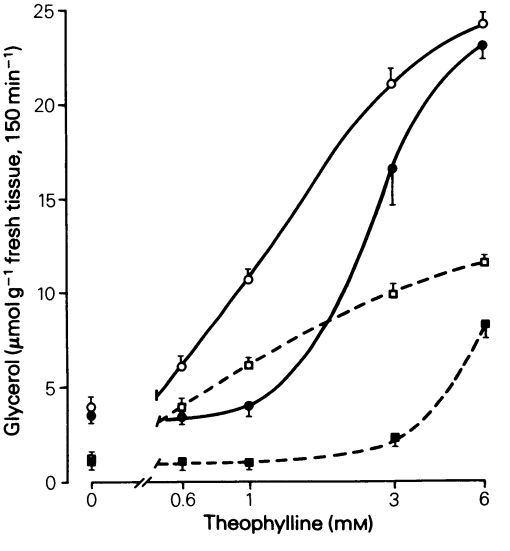

Figure 1 Effect of nicotinic acid on theophylline induced lipolysis in adipose tissue from rats of different ages. Epididymal fat pads from 6–7 week old rats in the absence (○) and in the presence (●) of 1 mM nicotinic acid. Epididymal fat pads from 16–20 weeks old rats in the absence (□) and in the presence (■) of 1 mM nicotinic acid. Adipose tissue was incubated in Krebs Ringer solution (pH 7.4) containing 2.5% albumin. Each value represents the mean ± s.e.mean of 8 determinations from 4 different experiments.

Table 4 Effects of nicotinic acid on theophylline-induced lipolysis in adipose tissue of rats of different ages

Drugs (mM)	*FFA* (μEq g^{-1} fresh tissue, .150 min^{-1})	*Glycerol* (μmol g^{-1} fresh tissue, .150 min^{-1})	*FFA: glycerol*
	6–7 weeks		
None	4.87±0.58	2.95±0.57	1.65
Theophylline 3	37.62±2.27	21.72±1.54	1.73
Theophylline 3 + NA 0.01	35.75±2.73	18.21±2.03	1.89
Theophylline 3 + NA 0.1	36.90±4.02	18.60±1.76	1.98
Theophylline 3 + NA 1	37.65±2.19	19.85±2.06	1.89
	10–12 weeks		
None	4.72±1.50	1.65±0.27	2.86
Theophylline 3	29.25±3.15	13.83±2.10	2.11
Theophylline 3 + NA 0.01	24.61±4.24	9.54±1.28	2.58
Theophylline 3 + NA 0.1	24.46±5.02	8.58±1.66*	2.85
Theophylline 3 + NA 1	23.11±4.36	8.08±1.46**	2.86
	16–20 weeks		
None	4.05±0.41	1.13±0.18	3.58
Theophylline 3	23.12±1.07	10.30±0.49	2.24
Theophylline 3 + NA 0.01	13.83±1.30***	7.04±0.34***	1.96
Theophylline 3 + NA 0.1	11.46±1.81***	4.29±0.73***	2.67
Theophylline 3 + NA 1	9.80±1.18***	3.82±0.45***	2.57

The age of rats is shown at the top of each group of data. Medium albumin: 2.5%. Each value is the mean ± s.e.mean of the results from 4 different experiments. FFA: glycerol ratio was calculated from the values shown in the table. The statistical significance of the difference from theophylline 3 mM is indicated as: *$P<0.05$; **$P<0.025$; ***$P<0.001$.

The reduction of FFA release was less marked and statistically not significant.

In adipose tissue from the oldest rats (16–20 weeks), 3 mM theophylline elicited a 9 fold increase in the rate of lipolysis, but the total amount of glycerol released from the tissue was again much lower than in the youngest animals (Table 4). Nicotinic acid now significantly inhibited the effect of theophylline even at the lowest concentration tested (0.01 mM). The release, both of FFA and glycerol induced by theophylline, was reduced by nicotinic acid to about the same extent and the effect was concentration-dependent (Table 4). The highest nicotinic acid concentration (1 mM) caused an inhibition of lipolysis of about 60%.

In the presence of theophylline no consistent changes in FFA: glycerol ratio were observed in relation to age or to the addition of nicotinic acid. As the activation of lipolysis caused by 3 mM theophylline was almost maximal, the antilipolytic effect of 1 mM nicotinic acid was also tested against a range of theophylline concentrations (0.6–6 mM). As shown in Figure 1, the submaximal activation of glycerol release elicited by 0.6 and 1 mM theophylline was abolished by nicotinic acid in adipose tissue from both 6–7 weeks and 16–20 weeks old rats. At 3 and 6 mM theophylline the antilipolytic effect of nicotinic acid was significant in the oldest animals, but not in the youngest.

Discussion

Rats have been the most commonly used species for studying the metabolic effects of nicotinic acid. In this species the aging process causes a number of changes in adipose tissue as far as fat cell number and size, and various metabolic activities are concerned (Gellhorn & Benjamin, 1965; Forn *et al.*, 1970; Holm *et al.*, 1975; Kardovà, Fabry & Vrana, 1978; Cleary, Brasel & Greenwood, 1979). Most of the changes in the parameters occur very rapidly within six months after birth, while from 6 to 24 months the rate of change is much lower (Cooper & Gregerman, 1976). Because of these reports we chose the particular three different age groups of rats for the present study.

The enlargement of rat fat pads during the first year of life is directly related to the increase of body weight (Cleary *et al.*, 1979) and is mostly due to adipocyte hypertrophy, while hyperplasia stops when rats reach a body weight of about 300 g, which is close to the weight of the 10–12 week old rats used in our experiments. Owing to these quantitative changes, particular emphasis will be placed on the FFA to

glycerol ratio, which is independent of the tissue weight. This parameter was markedly increased by age. In the young rats this ratio was lower than 3, suggesting that a rapid FFA re-esterification and/or oxidation occurs in the tissue. As the animals became young adult and then more mature, the ratio progressively increased to a value of 3 and even higher, indicating a decrease in the rate of FFA re-esterification and/or oxidation, possibly associated, at least in older animals, with an accumulation of diglycerides. This observation is in accordance with the age-related decrease of palmitic acid oxidation and incorporation into triglycerides (TGL) (both expressed per mg tissue) reported by Benjamin, Gellhorn, Wagner & Kundel (1961), albeit that Reardon *et al.*, (1973) found no significant change in the basal rate of FFA esterification (referred to number of cells) in fat cells from various age groups of 16 h fasted rats.

Independently of the age of the animals, nicotinic acid caused a small increase of FFA mobilization, with a concomitant reduction in basal glycerol release. As a result of these opposite changes, which are not statistically significant, the drug markedly enhanced FFA to glycerol ratio in a concentration-dependent manner. Thus nicotinic acid seemingly caused changes in the basal turnover of FFA, which superficially resembled changes with age. This point, however, needs to be clarified, because there is no direct proof for such an effect of nicotinic acid on FFA oxidation or partial hydrolysis of TGL. It has been shown that the drug does not alter the basal rate of FFA esterification in adipose tissue from normal rats, but that it stimulates this process in altered conditions such as fasting (Solyom & Puglisi, 1966) and alloxan diabetes (Östman, 1964).

Besides this possible influence of nicotinic acid on the basal turnover of FFA, the fact that the antilipolytic action of this drug strictly depends upon the age of the animals is of peculiar interest. Nicotinic acid did not counteract the effect induced by noradrenaline on TGL hydrolysis in adipose tissue from 6–7 weeks old rats. This was true when both high and low catecholamine concentrations were tested, thus excluding the possibility that the inhibitory effect of nicotinic acid might be masked by a supramaximal stimulation of lipolysis. Negative results were also obtained when the albumin concentration in the medium was doubled, in order to clarify whether the availability of binding sites for FFA released into the medium was a limiting factor for the full activation of lipolysis, thus influencing the effect of nicotinic acid.

Similarly in adipose tissue of the youngest rats nicotinic acid did not alter FFA and glycerol release stimulated maximally by 3 mM theophylline. In 10–12 weeks old rats the drug reduced the stimulation of lipolysis caused by theophylline but not by noradrenaline. The highest sensitivity of adipose tissue to the antilipolytic action of nicotinic acid was attained when the animals grew to an age of about 5 months. In this case the drug not only caused a marked inhibition of the effect of theophylline (see Figure 1 for detailed comparison with younger rats), but also partially antagonized the stimulation elicited by noradrenaline.

These results suggest that age or, alternatively, metabolic changes which occur during the aging process, is an important factor in the action of nicotinic acid in adipose tissue. Its antilipolytic effect is known to be mediated by cyclic AMP (Andersson, Harthon, Hedström & Lundholm, 1973; Kather & Simon, 1979). As rats grow older, hormone-induced adenylate cyclase activity in adipose tissue decreases, with a simultaneous increase of phosphodiesterase activity (Forn *et al.*, 1970; Giudicelli & Pecquery, 1978). As a result catecholamines would be expected to induce only a moderate increase of cyclic AMP level in older animals, possibly within the range in which cyclic AMP changes are fully coupled with those of lipolysis (Fain, 1977). In these conditions any decrease of the intracellular cyclic nucleotide content caused by nicotinic acid would result in an antilipolytic effect of the drug. In adipose tissue of young rats in which, due to the very high sensitivity of adenylate cyclase to external stimuli, a large accumulation of cyclic AMP occurs, nicotinic acid could still reduce the cyclic nucleotide level, but not below the limit which is sufficient for the full activation of lipolysis (Fain, 1977). On the other hand the fact that the antagonism elicited by nicotinic acid towards the lipolytic action of theophylline was more marked in the older rats indicates that a tight coupling exists between the site of theophylline action and the final lipolytic response.

There may be other explanations for the age-related increase of nicotinic acid action. For instance the fact that nicotinic acid causes a parallel inhibition of FFA and glycerol release only in the oldest rats, while in adipose tissue from younger animals its effect on FFA was weaker than its effects on glycerol, suggests that the changes induced by age on FFA turnover may also influence the action of nicotinic acid at this level.

Associated with the aging process are a number of alterations in the metabolic features of adipose tissue and nicotinic acid is known to have effects on all these features; for instance, it increases the rate of glucose utilization (Shafrir *et al.*, 1971) and favours its flow through the pentose cycle (Lee, Ellis & Sigal, 1961; Shafrir *et al.*, 1971), stimulates acetate incorporation into lipids (Lee *et al.*, 1961) and increases glycogen stores (Shafrir *et al.*, 1971) in adipose tissue. Thus there are many possible actions which singly or collectively could account for the effect of nicotinic acid.

It is intriguing to contemplate that a specificity might exist for this drug, by which it would prevent or counteract some potentially dangerous effects of aging, without altering the physiological equilibrium of young organisms.

This work was supported by grant C.N.R. no. 79.01884.04 and no. 81.00200.04.

References

ANDERSSON, R., HARTHON, L., HEDSTRÖM, M. & LUNDHOLM, L. (1973). Inhibition of cyclic AMP formation and lipolysis in rat adipose tissue by nicotinic acid. *Atherosclerosis*, **18**, 399–406.

BENJAMIN, W., GELLHORN, A., WAGNER, M. & KUNDEL, H. (1961). Effect of aging on lipid composition and metabolism in the adipose tissue of the rats. *Am. J. Physiol.*, **201**, 540–546.

BIERMAN, E.L. & ROSS, R. (1975). Aging and atherosclerosis. In *Atherosclerosis Reviews*. ed. Paoletti & Gotto, vol. 2, pp. 79–111. New York: Raven Press.

CARLSON, L.A. (1963). Studies on the effect of nicotinic acid on cathecolamine stimulated lipolysis in adipose tissue in vitro. *Acta Med. Scan.*, **173**, 719–722.

CARLSON, L.A. (1975). Serum lipoprotein composition in different types of hyperlipoproteinemia. In *Lipids, lipoproteins and drugs. Adv. exp. Med. Biol.* Vol. 63, ed. Kritchevsky, Paoletti & Holmes, pp. 185–199. New York: Plenum Press.

CARLSON, L.A. (1978). Nicotinic acid and inhibition of fat mobilizing lipolysis. Present status of effects on lipid metabolism. In *Drugs, lipid metabolism, and atherosclerosis. Adv. exp. Med. Biol.* Vol. 109, ed. Paoletti & Holmes, pp. 225–238. New York: Plenum Press.

CLEARY, M.P., BRASEL, J.A. & GREENWOOD, M.R.C. (1979). Developmental changes in thymidine kinase, DNA and fat cellularity in Zucker rats. *Am. J. Physiol.*, **5**, E508–E513.

COOPER, B. & GREGERMAN, R.I. (1976). Hormone-sensitive fat cell adenylate cyclase in the rat. Influence of growth, cell size, and aging. *J. clin. Invest.*, **57**, 161–168.

DIOGUARDI, N. & VERGANI, C. (1978). Introduction on human hyperlipoproteinemias. In *Human hyperlipoproteinemias. Principles and methods. Adv. exp. Med. Biol.*, Vol. 38, ed. Fumagalli, Ricci & Gorini, pp. 3–19. New York: Plenum Press.

DOLE, V.P. (1956). A relation between non esterified fatty acids in plasma and the metabolism of glucose. *J. clin. Invest.*, **35**, 150–154.

FAIN, J.N. (1977). Cyclic nucleotides in adipose tissue. In *Cyclic 3′–5′-nucleotides: mechanism of action*, ed. Cramer & Schultz, pp. 207–228. London: J. Wiley & Sons.

FORN, J., SCHÖNHÖFER, P.S., SKIDMORE, I.F. & KRISHNA, G. (1970). Effect of aging on the adenyl cyclase and phosphodiesterase activity of isolated fat cells of rat. *Biochim. biophys. Acta*, **208**, 304–309.

GELLHORN, A. & BENJAMIN, W. (1965). Effects of aging on the composition and metabolism of adipose tissue in the rat. In *Handbook of Physiology*, Sect. 5. *Adipose Tissue*, ed. Renold & Cahill, pp. 399–405. Washington: American Physiological Society.

GIUDICELLI, Y. & PECQUERY, R. (1978). β-adrenergic receptors and cathecolamine-sensitive adenylate cyclase in rat fat cell membranes: influence of growth, cell size and aging. *Eur. J. Biochem.*, **90**, 413–419.

GOLDSTEIN, S. & HARLEY, C.B. (1969). In vitro studies of age-associated diseases. *Fedn. Proc.*, **38**, 1862–1867.

HEPP, K.D., DIETZE, G. & WIELAND, O. (1971). Studies on the antilipolytic action of nicotinic acid. In *Metabolic effects of nicotinic acid and its derivatives*, ed. Gey & Carlson, pp. 357–366. Bern: Hans Huber Publ.

HOLM, G.B., JACOBSSON, B., BJÖRNTORP, P. & SMITH, U. (1975). Effects of age and cell size on rat adipose tissue metabolism. *J. Lipid Res.*, **16**, 461–464.

KARDOVÀ, L., FÀBRY, P. & VRÀNA, A. (1978). Effect of age, nutritional and hormonal factors on the cellularity of adipose tissue, In *Diabetes, obesity and hyperlipidemias*, ed. Crepaldi, Lefebvre & Alberti, pp. 149–157. London: Academic Press.

KATHER, H. & SIMON, B. (1979). Antagonistic effects of prostaglandin E_1 and nicotinic acid on the human fat cell adenylate cyclase. *Res. Comm. Chem. Pathol. Pharmac.*, **23**, 81–88.

KRISHNA, G., WEISS, B., DAVIES, J.I. & HYNIE, S. (1966). Mechanism of nicotinic acid inhibition of hormone induced lipolysis. *Fedn. Proc.*, **25**, 719.

LAMBERT, M. & NEISH, A.C. (1950). Rapid method for the estimation of glycerol in fermentation solution. *Can. J. Res.*, **28**, 83–89.

LEE, H.M., ELLIS, R.M. & SIGAL, M.V. (1961). Some insulin-like effects of nicotinic acid observed with isolated rat epididymal adipose tissue. *Biochem. biophys. Acta*, **49**, 408–410.

MASORO, E.J., BERTRAND, H., LIEPA, G. & YU, B.P. (1979). Analysis and extrapolation of age-related changes in mammalian structure and function. *Fedn. Proc.*, **38**, 1956–1961.

NASH, T. (1953). The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochem. J.*, **55**, 416–421.

ÖSTMAN, J. (1964). Effect of nicotinic acid on fatty acid metabolism of adipose tissue in alloxan diabetic rats. *Metabolism*, **13**, 675–680.

OTWAY, S., ROBINSON, D.S., ROGERS, M.P. & WING, D.R. (1971). The effects of nicotinic acid and of glucose on adipose tissue and heart clearing factor lipase activities and free fatty acid concentrations in the starved rats. In *Metabolic effects of nicotinic acid and its derivatives*. ed. Gey & Carlson, pp. 497–514. Bern: Hans Huber Publ.

REARDON, M.F., GOLDRICK, R.B. & FRIDGE, N.H. (1973). Dependence of rates of lipolysis, esterification and free fatty acid release in isolated fat cells on age, cell size, and nutritional state. *J. Lipid Res.*, **14**, 319–326.

RIFKIN, B.M., LA ROSA, J. & HEISS, G. (1980). Prevalence of hyperlipoproteinemia in selected north american populations. In *Atherosclerosis V*, ed. Gotto, Smith & Allen, pp. 264–267. New York: Springer Verlag.

ROTH, G.S. (1979). Hormone receptor changes during adulthood and senescence: significance for aging research. *Fedn. Proc.*, **38**, 1910–1914.

SHAFRIR, E., OREVI, M. & GUTMAN, A. (1971). Effect of nicotinic acid on carbohydrate metabolism in adipose tissue. In *Metabolic effects of nicotinic acid and its derivatives*, ed. Gey & Carlson, pp. 769–771. Bern: Hans Huber Publ.

SKIDMORE, I.F., SCHÖNHÖFER, P.S. & KRITCHEVSKY, D. (1971). Effects of nicotinic acid and some of its homologues on lipolysis, adenyl cyclase, phosphodiesterase and cyclic AMP accumulation in isolated fat cells. *Pharmac.*, **6**, 330–338.

SOLYOM, A. & PUGLISI, L. (1966). Effect in vitro of nicotinic acid on triglyceride synthesis in rat adipose tissue. *Biochem. Pharmac.*, **15**, 41–48.

WALTON, K.W. (1974). Hyperlipidaemia and the pathogenesis of atherosclerosis. In *Advances in drug research*. Vol. 9, ed. Harper & Simmonds, pp. 55–67. London: Academic Press.

(*Received February 9, 1983.*
Revised May 4, 1983.)