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Short-Term Infusion of Azelaic Acid *vs* Intralipid in Healthy Subjects Evaluated by Indirect Calorimetry

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ABSTRACT. Medium-chain dicarboxylic acids (MCDA) are usually considered byproducts of β-oxidation when Ω-oxidizable medium-chain monocarboxylic acids are accumulated, as in β-oxidation impairment. However, evidence exists of a mitochondrial and cytoplasmatic peroxisomial carnitine independent β-oxidation of these diacids. Our purpose was to evaluate whether MCDA could be used as source of calories. The metabolic response to intravenous administration of azelaic acid (AA) vs Intralipid (IL) was evaluated in six healthy overnight fasting male volunteers who received an infusion of 10 g of AA over 80 min and as a control 10 g of IL.

AA reached a peak concentration at 80 min, (589 ± 61 μg/ml) and was rapidly cleared from plasma (82 ± 5 μg/ml at 240 min).

Respiratory and metabolic parameters were evaluated by indirect calorimetry from the beginning of the infusion for 240 min. In both groups the CO_2 production (VCO_2) remained unchanged with no significant change from basal values. The O_2 consumption (VO_2 ml/min/m^2) increased over basal values reaching a peak at the end of the infusion in both groups (AA from 119.4 ± 16.9 to 143.0 ± 27.6; IL from 124.7 ± 16.8 to 152.3 ± 29.5). Respiratory quotient (RQ) consequently decreased significantly (AA from 0.85 ± 0.06 to 0.76 ± 0.06; IL from 0.89 ± 0.06 to 0.78 ± 0.03) and calories derived from lipids increased.

Metabolic rate (MR kcal/hr/m^2) showed a slight increase (AA from 34.0 ± 4.4 to 40.3 ± 6.8; IL from 35.9 ± 5.1 to 41.3 ± 10.5). There was no significant difference between AA and IL treatment in all measurements.

In the past dicarboxylic acids have been considered catabolic products, however our data suggest that the metabolic behavior of AA is very similar to long-chain triglycerides. AA is clearly oxidized and probably has further activating effects on the lipid metabolism in man. (*Journal of Parenteral and Enteral Nutrition* **14**:169–172, 1990)

Lipid infusion has been available over the past 30 years for parenteral use as a caloric source.[1–3] Different lipid mixtures have been used, the most widely accepted being the soybean emulsion (Intralipid:IL). The introduction of intravenous fat emulsion represents an important advance in total parenteral nutrition (TPN). The high caloric content of long-chain triglycerides (LCT) is not associated with excess carbon dioxide production, as is the case with hypertonic glucose solutions, which can be a valuable requisite with regard to respiratory distress.[4–6] Further experimental and clinical data confirm the significant preference for lipids as a fuel source in septic patients.[7] Nevertheless, in some particular conditions, in spite of higher utilization of lipid substrates, a low lipoprotein lipase activity may result in a diminished lipid clearance and consequent hypertriglyceridemia.[8] Among the studies concerning alternative substrates in TPN we have considered the possibility of utilizing medium-chain dicarboxylic acids (MCDA) as energy substrates and as a fuel source. The metabolic fate of these compounds is not entirely known.[9,10] Evidence exists of both a mitochondrial and cytoplasmatic peroxisomial carnitine independent β-oxidation.[11,12] Therefore MCDA would have the advantage of being readily available to the cell, not requiring hydrolysis prior to intracellular utilization; in addition, being water soluble they could be directly administered by a peripheral venous route. Among MCDA we chose C9 azelaic acid (AA), because it has already been safely used in humans. In a previous paper we reported a preliminary study of the response to infusion of AA in man and performed a stoichiometric analysis of the AA, comparing its characteristics to common caloric sources (Table I).[29] The purpose of the present study is to investigate in greater depth the metabolic response to AA infusion comparing it to IL infusion using the same subjects as a control of themselves. We wish to further clarify whether AA as well as being a byproduct of β-oxidation could also be considered a caloric source.

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MATERIALS AND METHODS

Subjects

Metabolic studies were performed in six overnight fasting healthy male volunteers (informed consent was obtained from subjects as well as authorization from the Human Investigation Committee). The mean age was 40 ± 4 years; mean weight was 75 ± 3 Kg.

The infused AA solution consisted in 500 ml of double distilled water containing 10 g of AA salified with NaOH, KOH, $Mg(OH)_2$ and $Ca(OH)_2$, in the proportion at which the Na, K, Mg, and Ca cations are normally present in serum, sterilized by Seitz filter before administration.

After an overnight fast, six subjects received, through

a peripheral vein, an 80-min infusion of AA.

After 1 week we performed control metabolic studies on only four of the six subjects of the first group as two volunteers were not available. Ten grams of Intralipid were infused in the same way after an overnight fast over 80 min. Finally after a week the same four subjects received after an overnight fast, a control saline solution infusion.

AA concentration in various compartments was measured using a Gas Liquid Chromatography (GLC Hewlett Packard 5890A) method on venous blood and urine samples.

Respiratory and Metabolic Analysis

Metabolic and respiratory changes were evaluated by indirect calorimetry performed according to a previously developed method[13] with a computerized monitoring system. The system included: a mass spectrometer (MA Statham Gould) to rapidly analyze oxygen, carbon dioxide and nitrogen concentrations, a pneumotachometer (Hewlett Packard 47304A) to measure inspiratory and expiratory flow, an analog-digital converter and a microcomputer (Hewlett Packard 1000 A/600) for signal analysis, computation and storage of derived variables.[14-16] The tidal volume, partial pressure of O_2, CO_2, and N_2, end tidal values, oxygen consumption (VO_2 ml/min/m^2) and carbon dioxide production (VCO_2 ml/min/m^2) were calculated according to conventional formulas; respiratory quotient (RQ) was obtained as VCO_2/VO_2 ratio, metabolic rate (MR Kcal/m^2/hr) was obtained by a previously published computational method.[17] Theoretical BEE was assessed in each patient according to the Harris-Benedict formula.[18] We also calculated the percentage of MR derived from lipidic, glucidic, and proteic substrates oxidation using VO_2, VCO_2, and urinary nitrogen loss in 24 hr. Urinary nitrogen excretion was measured on the 4-hr and 24-hr specimens from the beginning of infusion.[19]

Statistical Analysis

Data were submitted to variance analysis.

RESULTS

VO_2, VCO_2, RQ, and MR analysis are shown in Figure 1. The 24-hr nitrogen loss was 18.5 ± 3.5 (g/24 hr) in the AA group and 18.0 ± 4.2 in IL group. No statistically significant difference was noted between the 4- and 24-hr specimen.

The basal VCO_2 value was 101.2(±9.9) ml/min/m^2 in the AA and 110.7(±16.8) ml/min/m^2 in the IL group. Thus the two groups had similar basal values and remained essentially unchanged throughout the experiment reaching a maximum value of 113.4(±17.6) and 121.2(±22.2) ml/min/m^2, respectively; there was no statistically significant change from basal values nor any difference between the two treatments.

In accordance with other experimental designs the control saline infusion study showed no significant change in metabolic parameters during the time period of 240 min.

The basal VO_2 value was 119.4(±16.9) ml/min/m^2 in the AA and 124.7(±16.8) ml/min/m^2 in the IL group; both the AA and IL group showed an increase of VO_2 with a peak of 143.0(±27.7) ml/min/m^2 at 80 min and 152.3(±29.5) ml/min/m^2 at 120 min, respectively. The increase of VO_2 was thus obtained somewhat earlier in the AA-treated patients. This rise was thereafter maintained until the end of the study. There was no statistical difference between the two treatments during the entire experiment and the VO_2 increase did not reach statistical significance.

RQ reflected the VO_2 and VCO_2 pattern; basal values were 0.85(±0.06) (AA) and 0.89(±0.06) (IL) and decreased to 0.76(±0.06) at 80 min and 0.78(±0.03) at 120 min, respectively, showing a significant drop ($p < 0.05$), but without any significant difference between AA and IL.

Basal MR values were 34.0(±4.4) (AA) and 35.9(±5.1) (IL) Kcal/hr/m^2 and showed an increase to 40.3(±7.7) (AA) and 41.2(±10.5) (IL) at the end of the study, 240 min (not significant). AA, measured by gas-liquid chromatography, reached a peak concentration at 80 min, (589 ± 61 μg/ml) and was rapidly cleared from plasma (82 ± 5 μg/ml at 240 min). AA (6.5 ± 1 g) were found unchanged in urine during the 240 min of the study. After 240 min no more AA was excreted in the urine. The analysis according to De Weir equations[17] (Fig. 2*a*) showed a marked change in the quantity of calories derived from lipid oxidation during and after infusion. The percent of calories derived from lipids before the infusion was 26.9(±21.8) for AA and 15.8(±22.5) for IL and increased to above 50% in both groups ($p < 0.05$). Conversely the glucose oxidation (GOR, Kcal/hr/m^2) (Fig. 2*b*) decreased very rapidly from 12.6 ± 6.2 to 1.7 ± 9.8 in AA-treated and from 19.0 ± 8.2 to 9.2 ± 8.8 in IL-treated. Due to slight increase of MR the lipid oxidation rate (LOR) has increased from 9.2 ± 9.5 to 25.2 ± 15.1 and from 5.7 ± 10.0 to 18.9 ± 8.4, respectively, (Fig. 2*c*).

DISCUSSION

Azelaic acid is a straight saturated dicarboxylic acid with nine carbon atoms, having a molecular weight of

TABLE I
Stoichiometric comparison of substrates

Substrate	Oxidation products	O_2 mM/Kcal	CO_2 mM/Kcal	RQ	MW	Kcal/g	ATP/M	ATP/KCal	ATP/CO_2	Energy recovery
Glucose	$CO_2 + H_2O$	9.0	9.0	1.0	188	3.7	38	0.057	6.3	40.4
Palmitic A	$CO_2 + H_2O$	10.0	6.9	0.7	256	9.0	129	0.056	8.1	40.3
Soybean EM	$CO_2 + H_2O$	10.1	7.1	0.7	271	9.0	138	0.057	7.9	41.3
Azelaic A	Malonic A. $+CO_2 + H_2O$	9.6	6.4	0.7	188	5.0	49	0.052	8.2	38.2

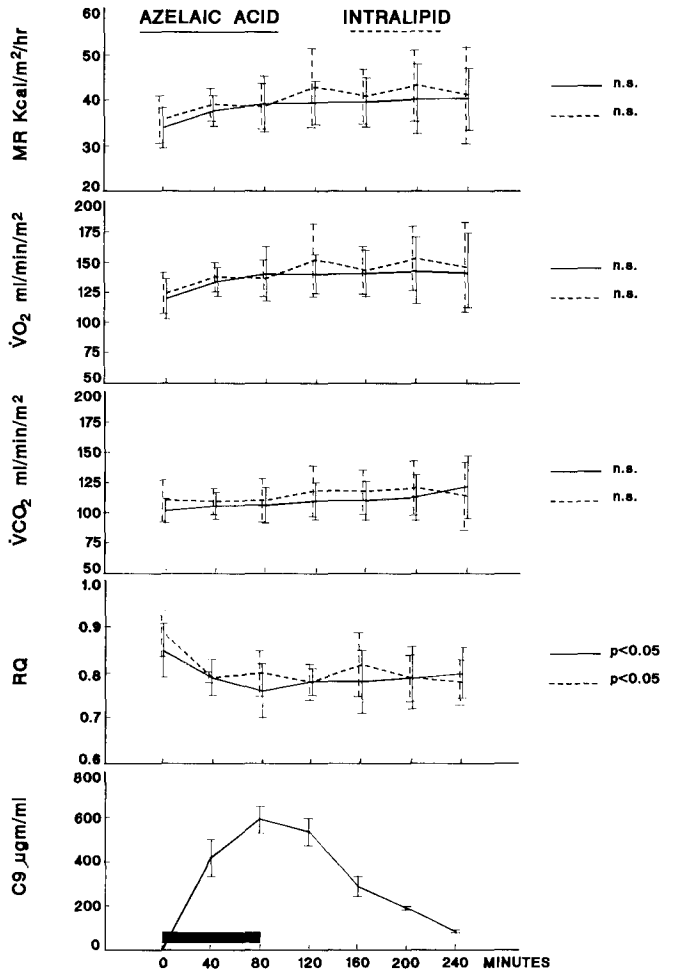

FIG. 1. Metabolic rate, oxygen consumption, carbon dioxide production, respiratory quotient, and plasmatic AA levels during the study period (means ± SD). The CO_2 production is virtually unchanged, while O_2 consumption and MR increase but do not reach statistical significance in both treatment groups. RQ decreases significantly.

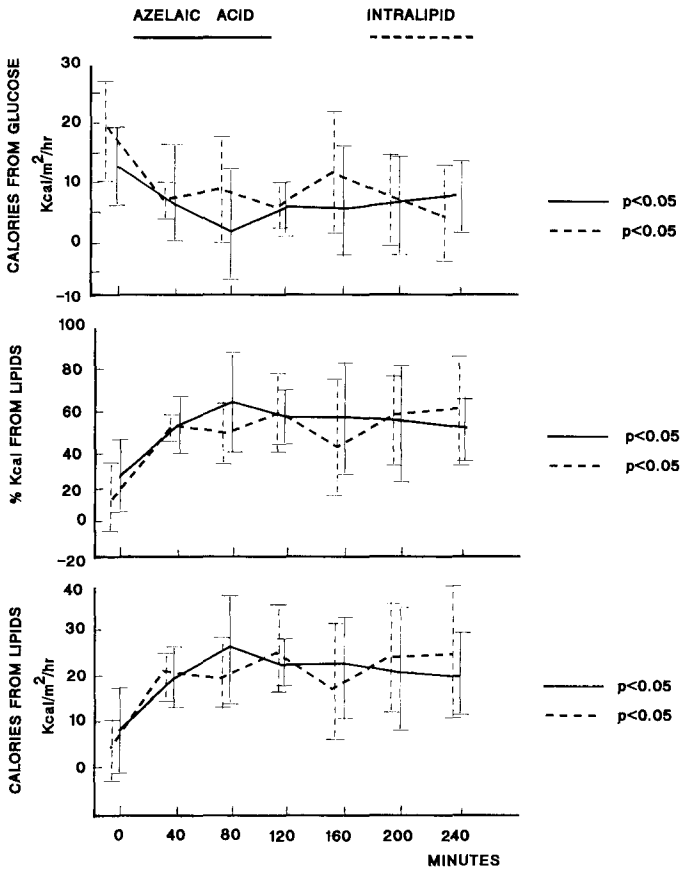

FIG. 2. The activation of lipolysis and depression of glucose utilization. *a*, The glucose oxidation rate, kcal/hr/m² in both AA-and IL-treated patients shows a statistically significant ($p < 0.05$) suppesssion. *b*, The percentage of the calories derived from lipids (mean ± SD) increases during the infusion and is maintained thereafter. *c*, The lipid oxidation rate kcal/hr/m² (mean ± SD) increases in both groups.

188.2 g. This diacid does not affect in any way the normal cells and does not exert either toxic or teratogenic effects in laboratory animals.[20] It can be β-oxidized in both humans and animals.[21] MCDA are CoA-activated and β-oxidized both in the mitochondria and in peroxisomes[22] using the same enzyme system as the monocarboxylic acids.[12,23] The transport of dicarboxylic acids across the mitochondrial membranes appears to be carnitine independent.[23–25] The β-oxidation of an odd-chain dicarboxylic acid, such as azelaic acid, gives malonil-CoA and acetyl-CoA as terminal products. While acetyl-CoA enters the Krebs' cycle to be completely oxidized to CO_2 and H_2O forming $FADH_2$ and $NADH^+H^+$, malonil-CoA cannot be further oxidized. Malonil-CoA represents, in fact, the starter of the synthesis of fatty acids.

The RQ, VO_2, VCO_2 values computed from the stoichiometric azelaic oxidation process (Table I) agree with the experimental data. Our results confirm the hypothesis that AA is oxidized in human subjects according to the stoichiometric data.[29] The idea that dicarboxylic acids are only by-products of β-oxidation through Ω-oxidation[26–28] and that they are used by cells only as a means to get rid of fatty acids in any condition in which β-oxidation is either defective or saturated appears to be rejected by our study.[9,18] Even if a large quantity of AA is excreted unchanged in the urine we can still observe a strong effect of AA on energy metabolism. The great loss in the urine observed in our study is probably due not only to the great water-solubility of the compound that readily passes through the glomerular barrier, but also to the modality of administration (rapid infusion rate, the choice of a salt form instead of a methylated form of AA). On the other hand the amount of AA that is not excreted in urine is rapidly cleared from plasma, and only small amounts of AA were found in plasma at the end of the study. This quantity had been actively removed from plasma and had passed into cells where a metabolic effect is expected if the AA is not a catabolic product but is an energy substrate. In fact, our study confirms that the behavior of AA was very similar to LCT. The activation of oxygen consumption without a rise in carbon dioxide production is compatible with the theoretical stoichiometric studies performed. During the infusion of AA and IL there is a shift toward lipids as substrate for oxidation as shown by calorimetric data; this shift is of quantitative importance and favors the conclusion that in our subjects AA has been oxidized to produce energy. The metabolic effect is even more striking if one considers that the caloric equivalent of AA is

only 5 kcal/g compared to the 9 kcal/g of IL. Thus normalizing the results by the administered calories a significant difference is evident since AA shows a greater metabolic activation compared to IL. The increase in MR would be roughly double after AA infusion. Whether this means an excess thermogenesis or an activation of lipolysis cannot be fully answered by the present study.

The amount of thermogenesis could not be computed as in our study we did not reach a constant plasma level of AA and consequently a plateau steady state. Another important aspect to be considered is the glucose oxidation rate expressed by the calories derived from glucose oxidation. As in our patients the protein catabolism can be considered stable over the short period of study the other major contributor to the calories consumed is glucose.

The glucose oxidation decreased in our subject very rapidly, furthermore our subjects, both IL and AA treated, underwent a slight increase of MR. Thus the lipid oxidation rate increased as absolute values and conversely there is a marked depression of the glucose utilization. This means that the administration of lipids, both LCT and MCDA, without a simultaneous infusion of glucose suppresses the glucose oxidation, an effect likely to be due to insulin suppression by lipid infusion.

Blood glucose level was unchanged through the study. The amount of calories derived from the stoichiometric analysis of AA was less than what was experimentally observed; the calories produced by the subjects have been derived from lipid oxidation; the glucose oxidation is suppressed: all this evidence suggests that AA has produced a catabolic effect of endogenous fat. A confirmation would probably need tracer studies on FFA turnover and long-term administration in combination with other substrates, eg, glucose. In conclusion we can state that AA is certainly an active metabolic product, it is clearly oxidized and probably has further activating effects on the lipid metabolism.

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