ENDOCRINE AND METABOLIC DIFFERENCES IN FORMULA-FED AND BREAST-FED INFANTS

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BY

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I. INTRODUCTION

There has been much interest in the differences between human breast milk and infant cow's milk formulas in the last decade. Lucas et al. (1) recently have reviewed the differences in the biochemical composition of both breast milk and cow's milk formulas. Advantages and disadvantages exist for both types of milk (2,3,4); one possible disadvantage and major controversial point is the influence of formula-feeding to obesity (5,6). Several different studies showed increased weight gain and increased skinfold thickness in formula-fed infants in comparison to breast-fed infants (7,8,9). However, neither the impact of these findings on the development of obesity in childhood and adolescence is clear, nor are the reasons for differences in growth development of breast and formula-fed infants always known. Overfeeding is a frequent reason for overweight in formula-fed infants (10). Metabolic and endocrine responses of the body to different milks may be another reason that contributes to increased weight gain in formula-fed infants (1) . However, there is little information on these responses to different types of milk-feeding in infants. A few studies about fuel metabolites and hormone levels were conducted recently. This report will review the impact of weight gain in infancy to weight development in childhood, adolescence, and adulthood. The effect of either formula-feeding or breast-feeding on anthropometric measurements and serum concentrations of selected metabolites and hormones will be included. A research plan will be developed to investigate the response of several hormones and fuel metabolites to either breast-feeding or formula-feeding.

II. REVIEW OF LITERATURE

A. Impact of increased weight gain in infancy to weight development in childhood, adolescence, and adulthood

Before discussing the relationship between weight gain in infancy to development of overweight and obesity in later years, the terms overweight and obesity will be defined. Obesity is defined as excessive accumulation of adipose tissue whereas overweight is defined as excessive heavyness (11,12). Frequently, obesity and overweight occur simultaneously but the latter may also refer to excessive muscularity in atheletes or to edema associated with several diseases. A range of 110 to 120 percent of standard weight per height is considered as overweight while more than 120 percent of standard weight per height is considered as obesity (13).

Obesity and overweight in infancy are serious problems particularly if obese infants and children remain obese in later life. Some investigators have found a positive correlation between obesity in childhood or adolescence and adulthood (14). Schlueter et al. (15) state that five to thirty percent of all children and adolescents in industrial societies are obese, and that seventy to eighty percent of them become obese adults. However, obesity in adulthood and obesity in the first year of life are less well correlated.

In contrast other studies have shown that no significant correlation exists between weight in infancy and weight in childhood, adolescence, and adulthood. Neyzi et al. (16) did not find any significant correlations between weight in infancy and weight in adolescence in 185 individuals. Melbin and Vuille (17) used weight gain in infancy as an indicator for obesity in childhood. The authors found that children who gained weight rapidly during infancy (> 97th percentile) reduced their relative weight between seven and ten years of age. Weight gain is an appropriate measurement for prediction of obesity in later life (18);

however, measurement of skinfold thickness gives a better estimate of the amount of adipose tissue in the body (12). Fifty percent of the adipose tissue stored in the body lies under the skin, and the thickness of a skinfold picked up at strategic sites indicates the amount of subcutaneous fat (12). Sites of skinfold measurement mostly used are biceps, triceps, subscapulor and suprailiac (12). Prader et al. (19) used skinfold measurements to compare the amount of adipose tissue of 300 children of normal weight at the age of 15 years with measurements taken within the first 39 weeks of life. The authors did not find a significant correlation between skinfold increment from 13 weeks to 39 weeks and absolute values at 15 years.

In contrast, Sveger et al. (20) found that 50 percent of children overweight in the first year of life remained so at the age of 2 1/2 years. Eid (21) conducted a follow-up study of physical growth of 224 children and found significant correlations between weight gain in the first six months of life and weight at six to eight years of age. The percentage of obese and overweight children was significantly higher in the group which had a rapid weight gain (> 90th percentile) in infancy than in the group which had an average or slow weight gain (50th percentile or less). Charney et al. (22) stated that 36 percent of infants whose weight exceeded the 90th percentile in the first six months of life were overweight as adults whereas only 14 percent of those infants who had average or less weight (50th percentile or less) in the first six months became overweight adults. Heald and Hollander (23) compared weights of adolescent obese and nonobese girls with their weights at one year of age. Obese girls had a more rapid weight gain in early life and a higher weight at one year of age.

These studies do not indicate that overweight infants necessarily become overweight in childhood, adolescence, or adulthood but they suggest that overweight infants are more susceptible to overweight or obesity in later years. This can partly be explained by the inability of the body to reduce the number

of adipose tissue cells once they are established (11). If the infant develops an increased amount of adipose tissue cells (hyperplasia) the potential for development of adipose tissue by incorporation of substrate in available adipocytes will increase (24). Therefore, development of adipose tissue cells should be controlled in early years.

B. Effects of Breast-Feeding and Formula-Feeding on Weight Gain and Body Composition in Infancy

The basic assumption is made that formula-fed infants gain more weight than breast-fed infants and therefore, are more susceptible to obesity (6). Several different studies give some evidence for this assumption. Neumann and Alpaugh (8) investigated birthweight doubling time of 254 normal infants which were either breast-fed or formula-fed. Infants were considered breast-fed if the only source of milk was from nursing for a minimum of three months. Infants double their birthweight normally between four and five months of age (8). Formula-fed infants doubled their birthweight earlier than breast-fed infants: 3.6 months versus 4 months ($P < 0.01$). A comparison to Harvard percentiles for mean group weight and length showed that formula-fed infants had weight gains in excess of length gain. Therefore, their rapid weight gain refers probably to a greater increase in adipose tissue than in breast-fed infants. In Taits' study (10) 19 percent of 21 breast-fed infants and 59.6 percent of 240 artificially fed infants had a weight gain above the 90th percentile at six weeks of age ($P < 0.001$). Forty formula-fed infants were selected from the original group to assess their energy intake. Diet histories showed that the mean intake was 135 calories/kg/day which is higher than the RDA's (115 kcal/kg/day) for infants between birth and six months of age. Thus overfeeding probably contributed to increased weight gain in formula-fed infants. Thorogood et al. (25) compared weight of 291 one year old infants with their weights at discharge from hospital after birth. Eighteen percent of the infants formula-fed at the time of discharge were overweight in comparison to only three percent of the infants

breast-fed at the time of discharge from hospital. Abrahams et al. (26) included 66 black families in a nutrition survey. Besides collecting dietary information, the researchers compared weight/height ratios and triceps skinfold thickness of adult individuals which were either breast-fed or formula-fed in infancy. The individuals were divided into three age groups: 5-16 years, 17-35 years, and 36 years and over. Those individuals over 36 years of age and who received formula feedings as infants had significantly higher values for weight/height ratios compared with those breast-fed in infancy.

In contrast to these studies, Yeung et al. (27) did not find significant differences in weight, length, head circumference and arm circumference between breast-fed and formula-fed infants during the first three months of life. However at five and six months values for weight and length were significantly greater in the formula-fed group. Measurements of two skinfold thicknesses (triceps and subscapular skinfold) and weight per length ratios were also significantly higher in formula-fed infants at five months compared to breast-fed infants. These differences were still there at six months but were not significant. HODGSON (28) compared mean weights of 301 infants which were either breast-fed for more than one month or not breast-fed at all. In this study, no significant difference was found in mean weights between these two groups at six months and one year of age. Townsend (7) found that weight gains of formula-fed infants were higher than weight gains of breast-fed infants (breast-fed for at least three months) during the first year of life but not after one year of age where breast-fed infants were heavier. Swiet et al. (29) investigated the effect of feeding habits on weight of 758 infants. The authors found that formula-fed infants weighed more than breast-fed infants at 6 months of age but the difference was not significant. Himes (9) concluded after a review of the literature that the difference in weight gain between breast-fed and formula-fed infants is small in the first three months of life but weight gain tends to be greater in formula-fed infants from three to six months. The differences

average from about 200 to 500 grams at the end of the first year.

The presented studies show that type of milk feeding has an influence on weight development. Overfeeding is one reason for increased weight gain in formula-fed infants (6,10). Differences in endocrine and metabolic responses to breast milk and infants formulas may be another possible explanation (30).

C. Effects of Breast-Feeding and Formula-Feeding on Fuel Metabolites and Hormone Levels in Neonates

Studies on endocrine and metabolic responses to formula- and breast-feeding have been conducted in neonates. These studies include the analysis of serum levels of insulin, glucagon, and some more recently investigated pancreatic and gastrointestinal hormones (30). The release of these hormones in response to feeding may play an important role in the adaptation to extrauterine nutrition in the neonate (31). Some of these gastrointestinal and pancreatic hormones have a trophic action on the intestine and stimulate its structural development and also the growth of the exocrine pancreas. They may also have an effect on the release of pancreatic hormones (32). Prior to reviewing the recent data on hormonal levels and serum metabolites in relation to type of milk feeding, the main functions of pancreatic and gastrointestinal hormones will briefly be presented.

1. Hormones

a. Insulin

Insulin is a polypeptide which is synthesized in and released from the beta-cells of the endocrine pancreas (33). Its action on adipose tissue is important when considering differences in subcutaneous fat in breast-fed and formula-fed infants. Insulin promotes lipogenesis and inhibits lipolysis. It brings

about the uptake of both glucose and fatty acids, which are necessary for triglyceride synthesis, and it inhibits the lipase found in adipose tissue, which is responsible for triglyceride break down. Insulin output is mainly controlled by blood glucose levels (34).

b. Glucagon

Glucagon, which is secreted from the alpha-cells of the endocrine pancreas, is also important in regard to body composition of infants because it stimulates catabolism of protein and triglycerides (34). In hypoglycemic state of the body, it increases glucose supply to peripheral tissues to provide substrate to brain, nervous system, muscles, and adipose tissue. Glucagon is not only secreted in response to low blood glucose levels but it can also be stimulated by several amino acids and fatty acids (34).

c. Pancreatic Polypeptide (PP)

PP is found in the peripheral area of the pancreatic islet cells and also in the pancreatic exocrine parenchyma (35). The role of PP in normal physiology has not yet been elucidated. Animal and in vitro experiments suggest that PP may have a growth-regulating effect on gut, pancreas, and liver (36). It is released in response to food intake, protein being the main stimulating nutrient (37).

d. Gastrin

Gastrin is produced in the stomach wall where it is absorbed and carried by the blood stream to the gastric glands, causing hydrochloric acid secretion (38). It stimulates the growth of gastric mucosa, increases gastric motility, and stimulates pancreatic enzyme production. Food with a high protein content causes gastrin secretion (11).

e. Gastric Inhibitory Polypeptide (GIP)

GIP is a linear peptide with 43 amino acids (39). Like gastrin, and enteroglucagon levels, GIP-concentration in small intestine increases after birth (32). GIP's primary physiological effect appears to be the control of insulin secretion and it stimulates the beta-cells of the endocrine pancreas (40). In addition, it inhibits gastric acid secretion (36). GIP is released from duodenum and jejunum in response to intraluminal digestion products. Fat and carbohydrates seem to be potent stimulants for its release (40).

f. Secretin

Secretin is a polypeptide which is found in its highest concentrations in both duodenum and jejunum (41). It stimulates the exocrine pancreas to secrete basic digestive juice which contains high concentrations of bicarbonate (HCO_3^-) and low concentrations of chloride (CL^-). It is released after a meal when acid chyme coming from the stomach enters the duodenum (11).

g. Motilin

Motilin is found in high concentrations in the small intestine and also in the circulation (42). It enhances pepsin production in stomach and regulates the rate of gastric emptying (36). Its secretion is decreased after feeding of glucose and a mixed meal (43). Increases of motilin have been reported after oral administration of fat.

h. Neurotensin

Neurotensin is a tridecapeptide that has been isolated from brain and from mucosa of small intestine (44). Its role in gastrointestinal tract is not well investigated. Neurotensin may have a stimulating effect on gastric emptying, and also on the release of pancreatic polypeptide. Neurotensin is released from the gastrointestinal tract into circulation following the

ingestion of a meal. It was found that oral fat is the most potent stimulus. of neurotensin.

i. Vasoactive Intestinal Polypeptide (VIP)

VIP is found in the brain and is also present throughout the gastrointestinal tract (45). It has a wide variety of actions which include vasodilation, gastric acid production, and stimulation of insulin release by causing hyperglycemia in the blood. No significant release of VIP was observed after a meal (36).

j. Enteroglucagon

Enteroglucagon has its highest concentration in ileum and colon (46). Its main functions are thought to be promotion of mucosal growth and delay of intestinal transit time. It is released in response to carbohydrates and long chain triglycerides in the intestine (36).

Serum levels of described hormones depend to a great extent on the amount and type of food ingested. Studies which investigated the response of these hormones to formula-feeding and breast-feeding in neonates will now be reviewed.

2. Endocrine and Metabolic Responses to Formula-Feeding and Breast-Feeding

Despite similar composition of breast milk and modified infant formulas some researchers have found differences in hormone levels and fuel metabolites in breast- and formula-fed neonates. Lucas et al. (30) compared plasma levels of insulin, glucagon, gastric inhibitory polypeptide, and some metabolites such as glucose, lactate, pyruvate, glycerol, and ketone bodies of 79 six-day-old term infants which were either formula- or breast-fed. The mean milk intake was similar in both groups. The composition of the adapted cow's milk formula which was given to the artificially fed infants is shown in comparison to

breast milk in table 1.

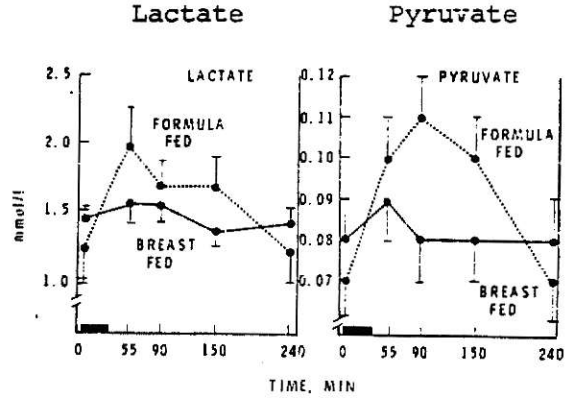
Table 1: The Composition of an Adapted Formula in comparison to Human Breast Milk

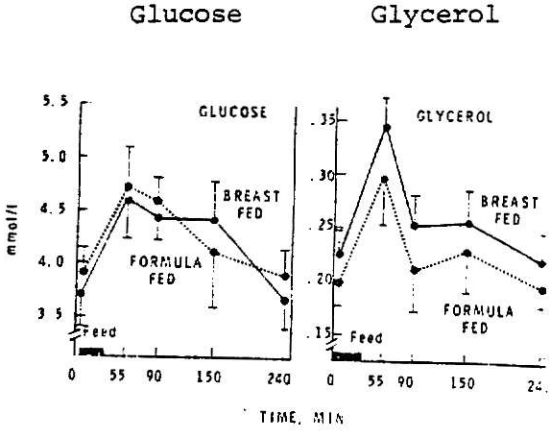
Nutrients	Adapted Formula	Human Breast Milk
Energy (kcal/100 ml)	70.0	69.0
Protein (g/100 ml)	1.80	0.90
Fat (g/100 ml)	1.45	4.50
Lactose (g/100 ml)	6.90	6.80

(Lucas et al., 30; Hambraeus, 47).

Protein content is higher and fat content lower in the adapted formula than in human breast milk. The fat content in this adapted formula is lower than in other commercially available formulas which have a fat content between 2.5 and 4 percent (48). Lucas et al (30) found that basal and postprandial levels of glucose, lactate, pyruvate, and glycerol were slightly different in breast-fed and formula-fed infants. These values are shown in fugure 1.

Figure 1: Effects of a feed on blood concentrations of lactate, pyruvate, glucose, and glycerol (Means ± SEM)





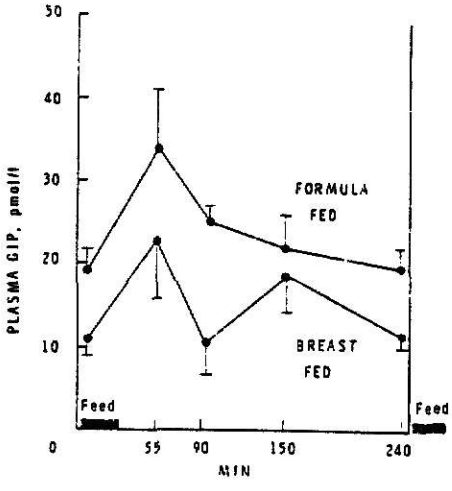
(Lucas et al., 30).

Serum levels of beta-hydroxybutyrate and acetoacetate did not change significantly after feeding in either breast-fed or formula-fed infants. However, the mean values of these two metabolites were higher in breast-fed infants than in formula-fed infants. Basal and postpradial levels of gastric inhibitory polypeptide were higher in

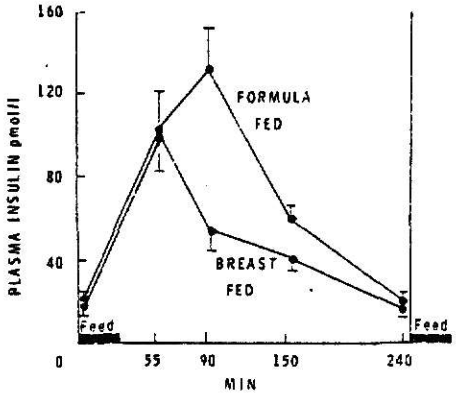
formula-fed infants; but this value was not significantly different from the value in breast-fed infants. The insulin increase following feeding was significantly higher and prolonged in formula-fed infants. Formula-fed infants had the insulin peak of 130 pmol/l at 90 minutes whereas breast-fed infants had a peak of 98 pmol/l at 55 minutes. The values for gastric inhibitory polypeptide and insulin are shown in figure 2.

Figure 2: Effect of a feed on plasma concentrations of GIP and insulin (Means & SEM)

GIP



Insulin



(Lucas et al. 30).

Lucas et al. (1) conducted a similar study which compared serum levels of insulin, GIP, and seven other gastrointestinal hormones in 77 six-day-old term infants which were either breast-fed or formula-fed. The mean volume ingested by the infants was similar. The formula used in this study ('Cow & Gate') is based on cows milk and butterfat without additional carbohydrates and is commercially available in Western Europe (49). Its composition is given in table 2.

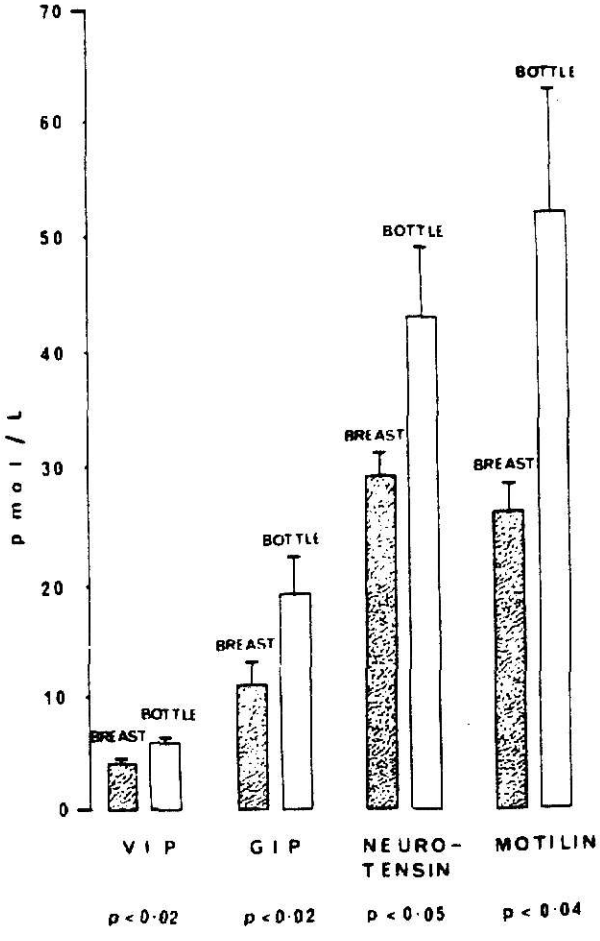
Table 2: Composition of an infant formula ('Cow & Gate') (gm/100 ml):

Protein	3.5
Fat	3.6
Carbohydrates	5.0

(Fomon, 49).

Fat and carbohydrate content are lower in this formula than in human breast milk (see table 1, p. 10), whereas the protein content is more than three times higher than in breast milk. This infant formula generally is prepared at home by addition of water and sucrose (49). The authors of this study found that the formula-fed group had significantly higher basal levels of GIP, VIP, motilin, and neurotensin than the breast-fed group (figure 3, p. 14).

Figure 3: Basal levels of gastric inhibitory polypeptide, vasoactive intestinal peptide, motilin and neurotensin in formula- and breast-fed infants. (MEANS ± SEM).



(Lucas et al., 1)

Basal levels of other hormones were similar in both groups and are shown in table 3.

Table 3: Basal levels of alimentary hormones in breast-fed and formula-fed infants (pmol/l)

	Breast fed	Formula fed	p
Secretin	3 ± 1	3 ± 1	NS
Gastrin	44 ± 4	40 ± 5	NS
Enteroglucagon	242 ± 26	210 ± 26	NS
Pancreatic polypeptide	28 ± 6	23 ± 6	NS

Values given as mean ± SEM.

(Lucas et al., 1)

Postprandial increases in serum concentrations of pancreatic polypeptide and enteroglucagon were significant in formula-fed infants if compared with basal levels; but not in breast-fed infants (Table 4).

Table 4: Plasma pancreatic polypeptide and plasma enteroglucagon responses in breast- and formula-fed infants

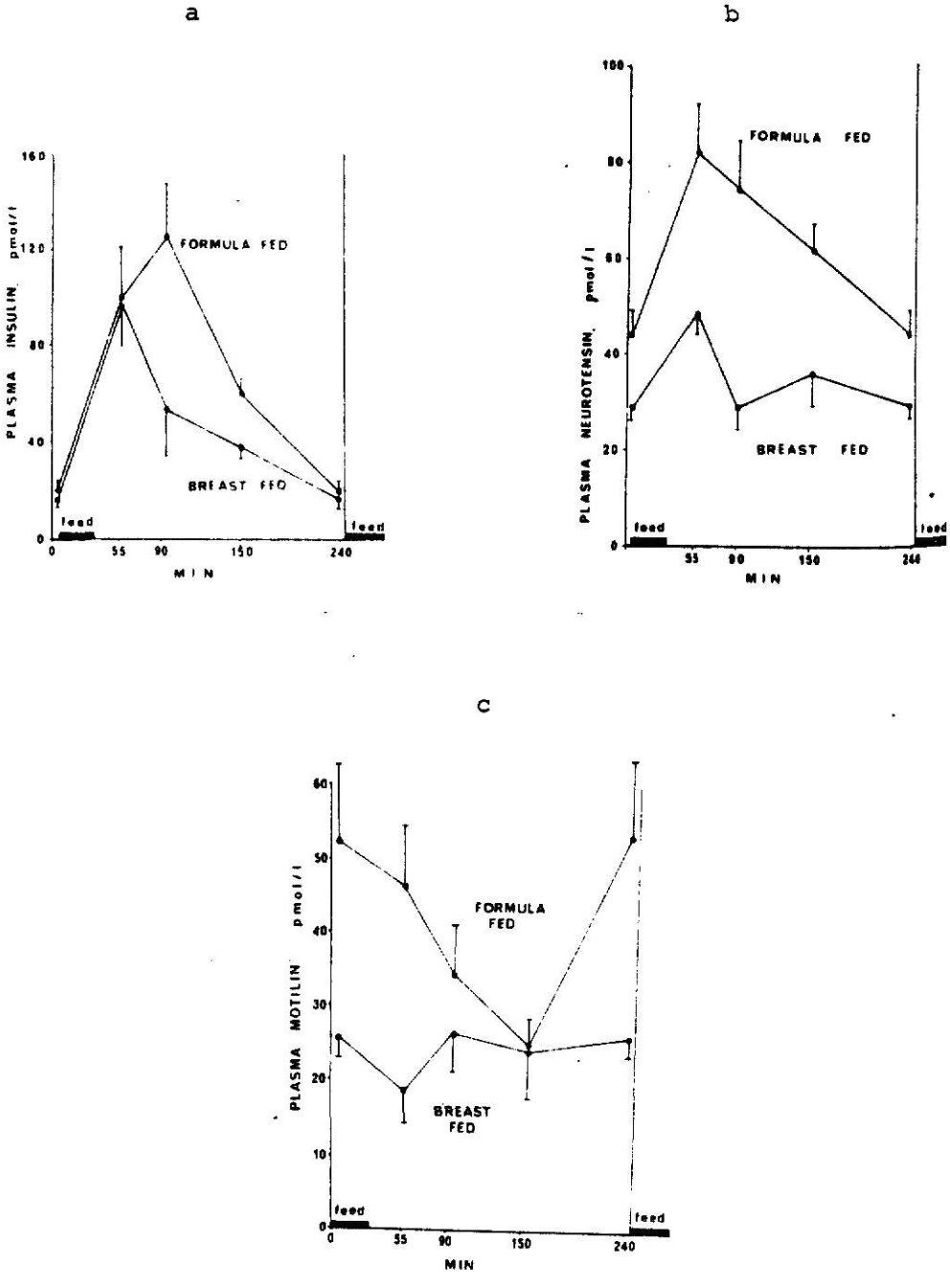
		Time (min)			
		0	55	90	150
Pancreatic polypeptide (pmol/l)	Breast fed	28 ± 6 (n = 10)	30 ± 5* (n = 12)	21 ± 3* (n = 12)	32 ± 6* (n = 9)
	Formula fed	24 ± 5 (n = 10)	19 ± 5* (n = 8)	20 ± 4* (n = 8)	49 ± 6 (n = 8) p < 0·01
Enteroglucagon (pmol/l)	Breast fed	202 ± 26 (n = 10)	225 ± 17 (n = 12)	193 ± 17 (n = 12)	237 ± 43 (n = 9)
	Formula fed	210 ± 26 (n = 10)	326 ± 67* (n = 8)	257 ± 46* (n = 8)	328 ± 45 (n = 8) p < 0·01

*NS
Results given as mean ± SEM.

(Lucas et al., 1)

Serum concentrations of insulin and neurotensin were significantly higher in formula-fed infants compared with breast-fed infants. The basal values of serum motilin were much higher in formula-fed infants than in breast-fed infants (53 vs. 25.5 pmol/l). Serum motilin levels decreased significantly after formula-feeding but not after breast-feeding (figure 4, p. 17). No changes were observed in plasma-concentrations of GIP, VIP, gastrin, and secretin after feeding in both groups.

Figure 4: Effect of a Feed on Plasma Insulin (a), Neurotensin (b), and Motilin (c). (MEANS ± SEM).



(Lucas et al., 1)

Bloom et al. (50) compared plasma levels of ten alimentary hormones in 72 six-day-old term infants. Thirty-three infants received breast milk and 39 infants received a modified cows milk formula. The infants received the same formula as the infants in the study of Lucas et al (1). As in the study presented before, formula-fed infants had significantly higher basal levels of motilin, neurotensin, vasoactive intestinal peptide, and gastric inhibitory polypeptide. Postprandial serum concentrations of insulin and neurotensin were larger in formula-fed infants. Elevations of enteroglucagon and pancreatic polypeptide, and depression of motilin were observed in response to formula feeding but not in response to breast feeding. Serum concentrations of glucagon, gastrin, and secretin were identical in the two groups. However, data of hormone concentrations observed in this study are not available. These studies show that basal and postprandial levels of gastrointestinal and pancreatic hormones are different in formula-fed and breast-fed infants. The reasons for these differences are not known. However, several factors may be involved in the production of hormonal differences in neonates depending on whether they were breast-fed or formula-fed.

The amount of food has an influence on hormone responses (50, 1). At the age of six days, when the study was conducted, the milk intake was similar in both groups of infants; however, breast-fed infants probably had a lower milk intake during the first days of life while lactation was established. This may have resulted in a slower development of the gastro-intestinal tract, and thus in a lower hormone production in breast-fed infants.

Food composition may also be important. The significant increase in PP levels after feeding may be caused by the higher protein content of the formulas used in the studies of Lucas et al. (1) and Bloom et al. (50) since PP release is stimulated by protein in the intestine (37). The increase of motilin levels in the breast-fed group in the study of Lucas et al.

(1) may be explained by the higher fat content of human breast milk in comparison to the used infants formula since motilin release is stimulated by oral administration of fat (43). It is difficult to explain the increased levels of other hormones in the formula-fed group by differences in milk composition; fat stimulates the release of GIP, and neurotensin (36); but the levels of these hormones were higher in formula-fed infants which received a formula with little fat content (1,30,50).

Gastric emptying rate can be influenced by the composition of food (39) and this in turn has an effect on gastrointestinal hormones. Fat is the most potent inhibitor of gastric emptying rate (34). Since the fat content of the modified formula, which was used in the study of Lucas et al. (30), was lower than the fat content of breast milk (see table 1, p. 10), gastric emptying rate may have been higher in formula-fed infants. Increased gastric emptying rates cause greater postprandial rises of plasma insulin, gastric inhibitory polypeptide, and glucose (39). This may explain the elevated insulin and gastric inhibitory polypeptide levels in the formula-fed infants.

The high ketone body concentration in breast-fed infants may be caused by a high rate of lipolysis reflecting low insulin levels (30). However, if lipolysis was higher in breast-fed infants than in formula-fed infants, glycerol levels should also be higher; but glycerol concentrations were only slightly different in both groups. Elevated ketone body levels in breast-fed infants may be explained by another mechanism. Warsaw and Curry (3) compared serum carnitine and ketone body concentrations in 11 breast-fed and 11 formula-fed infants. The authors found increased levels of these metabolites in breast-fed infants. They suggest that the higher serum carnitine concentrations could reflect the somewhat higher concentrations of carnitine in breast milk in comparison to infant formulas used in this study, and also a higher bioavailability of carnitine in breast milk. Carnitine is an essential carrier for acylgroups across the mitochondrial

membrane to sites of oxidation, and therefore has a central role in the mitochondrial oxidation of fatty acids (3). Thus, it is possible that ketone bodies were higher in breast-fed infants because increased carnitine availability leads to increased fatty acid oxidation, to excess of acetyl coenzyme A, and to ketone body formation. The differences in ketone body concentrations are interesting because ketone bodies inhibit glucose uptake and make tissues less sensitive to insulin (34). Since triglyceride synthesis in adipose tissue depends on insulin promoted glucose uptake, triglyceride synthesis and accumulation of adipose tissue could be somewhat deminished in breast-fed infants.

The greater insulin response to formula feeding is particularly interesting when considering that insulin promotes lipogenesis. Formula-fed infants, which had high insulin concentrations tend to gain more weight than breast-fed infants (1,30,50). One explanation for increased insulin levels after formula feeding may be a higher concentration of branched chain amino caids in the blood induced by the cows milk formula (1). Branched chain amino acids stimulate insulin release. Increased insulin secretion could also be explained by elevated levels of gastrointestinal hormones in formula-fed infants (51). Especially gastric inhibitory polypeptide stimulates insulin release (1).

In summary, several different reasons have been presented which may be responsible for greater weight gain and elevated hormone levels in formula-fed infants compared to breast-fed infants. However, differences observed in hormone and metabolite levels in formula- and breast-fed infants are most likely due to differences in nutrient composition of the milks.

Further research will clarify the influence of formula- and breast-feeding on hormone levels and metabolite concentrations in infants. Research in this area only included six-day-old neonates; therefore, further research should include infants who are older than six days of age to find out whether

differences will persist in older infants. A cow's milk formula should be used which has a composition similar to human breast milk. This will minimize differences in nutrient composition which may affect hormone and metabolite levels. Results should be related to body weight and body composition to gain knowledge about a possible connection between hormonal status and the development of overweight in infants. A research plan will now be presented.

III. RESEARCH PROPOSAL

A. Objective

The objective of this study is to determine whether differences in hormone levels and fuel metabolites between breast-fed and formula-fed infants persist up to the age of 3 1/2 months. Lucas et al. (1,30) and Bloom et al. (50) found that serum concentrations of gastrointestinal hormones and fuel metabolites were different in six-day-old infants depending on the type of milk feeding. Anthropometric measurements will show whether differences in endocrine state are related to physical development of infants.

B. Specific Aims

The specific aims for this study are as follows:

1. To assess the concentration of fuel metabolites such as glucose, glycerol, and ketone bodies, in formula-fed and breast-fed infants at two, eight, and fourteen weeks of age.
2. To assess serum concentrations of hormones such as gastric inhibitory polypeptide (GIP), insulin, thyroxine, growth hormone (GH), and somatomedin in formula-fed and breast-fed infants at two, eight, and fourteen weeks.
3. To assess anthropometric measurements which will include evaluation of weight and length, and measurement of skinfold thickness at three sites (triceps, subscapular, and suprailiac) at the age of two, eight, and fourteen weeks.

C. Rationale

Several different studies have investigated weight development or hormone and metabolite concentrations in blood in formula-fed and breast-fed infants.

Neumann and Alpaugh (8) and Taitz (10) found that formula-fed infants had higher weight gain than breast-fed infants. Bloom et al. (50) found higher levels of GIP and insulin in six-day-old artificially fed neonates when compared to breast-fed neonates. In addition, Lucas et al. (1,30) showed that levels of some metabolites (glucose, glycerol, ketone bodies) were slightly different in formula-fed and breast-fed infants. However, no study has been conducted which evaluates the relationship of weight gain and skinfold thickness to serum concentrations of hormones in infants older than six days of age fed different types of milk.

The analysis of several metabolites (glucose, glycerol, ketone bodies) will be included in this study because these metabolites are involved in lipid metabolism, and their assessment will give some indication for the rate of catabolic and anabolic processes which have an influence on body composition. Glucose stimulates insulin secretion, which in turn increases triglyceride synthesis. Glucose is also an important substrate of lipogenesis (34). Glycerol and ketone bodies are products of lipolysis and their serum concentrations give some indication for the rate of triglyceride break down (33). Lucas et al. (30) found that serum levels of these metabolites were higher in breast-fed than in formula-fed infants; thus breast-fed infants may have an increased rate of lipolysis.

Several hormones such as GIP, insulin, thyroxine, GH, and somatomedin will be analyzed because they have a great impact on metabolism and growth pattern. GIP stimulates insulin release and may be responsible for increased insulin levels found in formula-fed infants (1,30,50). The lipogenetic effect of insulin may be important when considering weight differences in infants.

GH and thyroxine (tetraiodthyronine) also are important in relation to weight differences in infants (34). GH stimulates fatty acid oxidation which may lead to increased ketone body formation. It also raises blood glucose concentration. Thyroxine increases glucose absorption from the intestine and

causes elevation of blood glucose concentrations. If thyroxine levels are chronically high, insulin release will be stimulated continually (34).

Somatomedins are small peptides which have anabolic, insulin like effects on muscle and adipose tissue (52). They enhance cell multiplication and stimulate cartilage proliferation. Growth hormone is necessary for somatomedin synthesis in the liver, and many effects of growth hormone are probably mediated by somatomedins (53). Somatomedins secretion also is regulated by insulin and nutrition. Insulin deficiency causes a decrease in somatomedin levels (52,54). Dietary protein appears to be important in both maintaining somatomedin activity and allowing somatomedins to stimulate cartilage metabolism (54). This may be important when considering differences in protein content of infant formulas and breast milk (47) as well as differences in insulin levels between breast-fed and formula-fed infants (1,30,50).

These hormones can possibly contribute to differences in physical development between breast-fed and formula-fed infants.

Anthropometric measurements will be taken because they provide the best index of growth status in infants and the degree of leanness and fatness at all ages (55).

D. Methodology

1. Experimental design

Forty-six fullterm healthy infants will be studied at the age of two, eight, and fourteen weeks. Male and female infants will be included, and sex differences will be taken into consideration when measurements are evaluated. The infants will be selected randomly through the Nutrition Program for Women, Infants, and Children (WIC), local physicians, and through employees and students of the university. The mothers will be contacted 6-8 weeks before delivery to find out how they plan to feed their infants (breast-

feeding or formula-feeding) and to train them for participation in the project. One group of 23 infants will receive human breast milk for 14 weeks and another group of 23 infants will receive a modified infant formula for the same time period. Enfamil[R], which is a commercially available formula prepared from non-fat cows milk, vegetable oils and added lactose (49) will be fed to the infants in this group. The composition of this formula is very similar to the composition of human breast milk as is shown in table 5.

Table 5: Average composition of human breast milk and one modified infant formula per 100 mls

		Human Breast Milk	Enfamil
Energy	(kcal)	69.0	70.0
Protein	(gm)	0.9	1.5
Fat	(gm)	4.5	3.7
Lactose	(gm)	6.8	7.0

(Hambraeus, 47; Reina, 56).

The energy intake of the infants will be recorded after each feeding. A record form will be provided to the mothers for this purpose (appendix A). Mothers of formula-fed infants will record the milliliters of prepared formula ingested by their infants. Mothers of breast-fed infants will weigh their infants before and after each feeding to record the difference in weight as amount of milk ingested by their infants (57). Since this test weighing and the weekly weighing for weight control in both groups of infants requires frequent weighing at home a balance will be provided to each mother.

A training program before delivery will instruct mothers about weighing methods, dietary record keeping, weight charts, and course, purpose, advantages, and disadvantages of the project. In addition, mothers will gain initial training experience in the hospital prior to discharge. Follow-up home visits will be made weekly to provide guidance in data recording.

Recumbent length and weight will be measured at each age (i.e. 2, 8, 14 weeks) when blood samples will be taken. The mean values of infants in the breast-fed and the formula-fed group will be related to standards of the National Center for Health Statistics (NCHS) to identify the number of underweight, normal, and overweight infants in each group (58). Subcutaneous fat will be evaluated by using Harpenden skinfold caliper. The fatfold thickness will be measured at three sites (triceps, subscapular, and suprailiac), since subcutaneous fat can be distributed in an uneven way, from one site to the other (59). The measures should be taken at the right side of the body in all infants.

Venous blood samples will be obtained from each infant from the back of the hand or the arteriell vein in the arm by means of an open-needle technique (1). Blood samples will be taken before feeding and 60, 90, and 150 minutes after the beginning of feeding to determine basal and postprandial levels of hormones and metabolites. The appropriate methods for preparation of blood samples immediately after withdrawing and for storage of the samples are given by Aynsley-Green et al. (32) and Davidson and Henry (60).

Concentrations of glucose, glycerol, acetoacetate, and beta-hydroxybutyrate in whole blood will be determined enzymatically (61). The amounts of blood needed for all four measurements together are very small (0.2 ml) (61).

Concentrations of gastric inhibitory polypeptide (GIP), insulin, growth hormone, and somtaomedins will be determined by radioimmunoassay (RIA).

The competition of a constant amount of labeled hormone with varying amounts of unlabeled hormone for the binding sites of a limited amount of antibody represents the principle underlying radioimmunological hormone estimation (62). By selecting suitable concentrations of labeled hormone and antibody it is possible to establish a system whereby the proportions of antibody-bound and free hormone depend on the concentration of unlabeled hormone.

Since it is possible to purify GIP (63), radioimmunoassay could be applied for GIP concentrations in serum. Bloom (64) developed a RIA-method for GIP by using a guinea pig antibody raised against GIP. Porcine GIP was used for iodination.

A RIA for insulin is described by Loeffler and Weiss (65). The separation of free and antibody-bound insulin will be obtained by absorption of the free insulin to dextran-coated charcoal. This method shows a good reproduceability and practicability. Only 10 micro liters are needed (66).

The only available method capable for measuring plasma levels of human growth hormone is the RIA (62). A double antibody RIA technique has been described by Morgan (67). As little as 1 milli micro gram in plasma can be detected with this method.

Somatomedin-C will be analyzed with a specific RIA described by Furlanetto et al. (68). Somatomedin-C can be detected using as little as 0.25 micro liters of whole blood. The method of determination of total thyroxine is based on the principle of competitive protein binding (69, 62). This assay is specific for thyroxine. Neither inorganic nor organic iodine contaminants interfere with this method (62). Total thyroxine can be determined using as little as 1 ml serum.

Since only 20 ml blood can be drawn from infants in the first three months of life at one time laboratory methods selected must use small amounts of blood or serum (70). The described methods require 8 ml blood each time of blood sampling and thus, comply with this requirement.

2. Pitfalls and Limitations

Since hormone levels are influenced by factors other than nutrition, precautions have to be taken when interpreting results. For example insulin is stimulated by a variety of hormones such as glucagon, growth hormone, adreno corticothophe hormone (ACTH), glucocorticosteroids, thyroxine, and gastric inhibitory polypeptide (57). Growth hormone levels vary greatly with state of wakefulness and physical activity (60). Values of hormone and metabolite concentrations depend also on the method of assay, fluctuations throughout the day and between individuals, and on the site from where blood samples are taken (57). Therefore, appropriate standards have to be selected. Individual values of metabolites and hormones may vary over a wide range and still be normal for the particular individual. Some difficulty may arise in locating parents who agree to participate with their infants because regular weighing and record keeping as well as regular visits of the mothers to the research unit may cause additional stress. The frequent blood sampling may cause some discomfort for the infants.

Another problem may be the accurate record keeping but the planned training program should minimize this difficulty.

Results may be influenced by deviation of milk intake in the research unit from milk intake at home because infants may be disturbed by change of environment and sampling procedures.

3. Human Subjects and Patient Consent

The experimental design and protocol will be submitted to the University Committee on Research Involving Human Subjects for review. Both parents will be required to sign an informed consent form which will provide a non-technical description of the purpose of the research program and its potential

risk and benefits (informed consent, App. B).

4. Possible Hazards and Benefits to the Participants

a. Blood loss: Laboratory methods have been selected which require small amounts of blood. Blood sampling will be kept to a minimum and will not exceed safe levels based upon the infants body weight.

b. Venipuncture: Catheters will be inserted in one hand or arm and may cause pain, irritation, or infection. The hazards will be carefully monitored and appropriate action taken should it become necessary.

c. No immediate benefit will be derived by either the infant or parent as a result of their participation in this study. Their participation however, will yield valuable information regarding infant feeding practices and potential effects on infant health.

5. Statistical Analysis

The experimental design for this study is a 2x3 factorial: 2 modes of feeding (i.e. breast- vs. bottle) and 3 age groups (i.e. 2 wks, 8 wks, 14 wks). The analysis of variance for two sample evaluation (t-test) will be used. (Personel communication with d. Dayton, Stat. Dept. KSU). Twenty-three infants in each group should be sufficient for determining a statistical difference of at least 20 p mole/dlin in post prandial insulin levels. Dr. Arthur Dayton in the Department of Statistics at Kansas State University will supervise the statistical analysis of data.

6. Significance

This research will expand the knowledge about the impact of feeding pattern in infancy on hormonal status and physical development of infants 2 - 14 weeks of age. Findings will show whether differences in these parameters which were observed in six-day-old infants (1,30) will persist up to an older age. Correlating feeding pattern and hormonal status to growth development of infants may give some insight to the problems of overweight in infancy and its relation to food intake.

SUMMARY

There has been much interest in the differences between human breast milk and infants cow's milk formulas in the last decade. Both types of feeding have advantages and disadvantages. One possible disadvantage and major controversial point of discussion is the influence of formula-feeding to obesity. Several studies showed that the type of milk fed has an influence to weight development of infants. Formula-fed infants tended to gain more weight and also to have increased skinfold thicknesses in comparison to breast-fed infants. Overfeeding is a frequently stated reason for overweight in formula-fed infants. Metabolic and endocrine responses of the body to different milks may be another reason that contributes to increased weight gain in formula-fed infants. A few studies have been conducted recently regarding fuel metabolites and hormone levels in six-day-old formula-fed and breast-fed neonates. These studies show that type of milk feeding has an influence on basal and postprandial serum levels of gastrointestinal and pancreatic hormones. The most striking finding was an elevated and prolonged response of serum insulin to formula-feeding. This may be explained by amount and composition of the milk as well as by levels of the hormone gastric inhibitory polypeptide which stimulates insulin secretion. The greater insulin response to formula-feeding may have an effect on weight gain and development of adipose tissue in infants since insulin is a lipogenic hormone.

A research plan is presented which will investigate the response of several hormones and fuel metabolites to either breast-feeding or formula-feeding. Anthropometric measurements will be taken to provide information about the growth status of infants and the degree of leanness and fatness. Correlating type of milk feeding and hormone status to growth development of infants may give some insight to the problems of overweight in infancy and its relation to food intake.

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APPENDIX A

Record Forms for Energy Intake

1. Breast-Fed Infants

NAME: _____

DATE: _____

TIME (HRS)	WEIGHT BEFORE FEEDING	WEIGHT AFTER FEEDING	MILK INTAKE (gm) (wt. after feeding -wt. before feeding)	CALORIE INTAKE (gm x 6.9 kcal)

2. Formula-Fed Infants

NAME: _____

DATE: _____

TIME (HRS)	AMOUNT OF MILK IN BOTTLE BEFORE FEEDING (ml)	AMOUNT OF MILK IN BOTTLE AFTER FEEDING (ml)	AMOUNT OF MILK INGESTED (ml) (ml after feeding -ml before feeding)	CALORIE INTAKE (ml x 7.0)

APPENDIX B

INFORMED CONSENT

I, _____, have agreed to participate with my infant, _____, in a study designed to evaluate the influence of type of milk feeding on blood levels of hormones and metabolites as well as to investigate the impact of type of milk feeding on physical development. I understand that my infant and I are free to withdraw without prejudice from this study at anytime.

Participation will require the following:

A. Physical Measurements

1. Body weight -- taken directly after delivery and on a weekly basis by the mother as well as during the experimental trials at two, eight, and fourteen weeks of the infants age.

2. Body height -- taken directly after delivery and during the experimental trials at two, eight, and fourteen weeks of the infants age.

B. Blood Sampling

1. Insertion of a catheter in the back of the right hand or the vein of the right arm before feeding and 60, 90, and 150 minutes after each feeding at two, eight, and fourteen weeks.

C. Maintaining Records Of:

1. The infants weight.

2. The amount of milk fed to my infant each time.

D. Training Program of Parents and Home Visits

1. Participation in a two hour training program (weighing and record keeping) at the research unit six weeks prior to delivery.

2. Supervised weighing and record keeping during the first days after delivery in the hospital.

3. Home visits of the investigator to guide parents in the performance of above described procedures.

E. Duration of:

1. Breast-Feeding for fourteen weeks, or

2. Formula-Feeding (Enfamil^{R}) for fourteen weeks.

The possible hazards to my infant during this study are outlined as follows:

1. Blood Loss: Laboratory methods have been selected which require small amounts of blood. Blood sampling will be kept to a minimum and will not exceed safe levels upon the infants body weight.

2. Venipuncture: A small venous catheter will be inserted in the right hand or arm and may cause pain, irritation, or infection. These risks are minimal and appropriate precautions will be taken.

Clinical judgement will at all times be used so as not to endanger my infants life or health. All precautions, both clinical and biochemical, will be taken to ensure my infants welfare.

I understand that my infants name and any study results connected with her/his name will remain confidential. I will have an opportunity at the end of the study to find out the conclusions. The generalities of the study have been explained to my satisfaction at this time, but I will feel free to ask questions regarding this study at any time.

I understand that no immediate benefits will be derived by either my infant or myself as a result of participation in this study. Participation, however, will yield valuable information regarding infant feeding practices and potential effects on infant health.

I have read and understand the above statement. I hereby voluntarily consent to cooperate in this study, and I agree with the participation of my infant.

Date	Signiture (Father,Mother)
	Witness

I have explained the above to the participants parents on the date stated on this Informed Consent.

Witness	Investigator
	Date

METABOLIC AND ENDOCRINE RESPONSES TO FORMULA-FEEDING AND BREAST-FEEDING

BY

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AN ABSTRACT OF A MASTER'S REPORT

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Foods and Nutrition

KANSAS STATE UNIVERSITY
Manhattan, Kansas
1983

ABSTRACT

There has been much interest in the differences between human breast milk and infant cow's milk formulas in the last decade. Both types of feeding have advantages and disadvantages. One possible disadvantage and major controversial point of discussion is the influence of formula-feeding to obesity. Several studies showed that the type of milk feeding has an influence to weight development of infants. Formula-fed infants tended to gain more weight and also to have increased skinfold thicknesses in comparison to breast-fed infants. Overfeeding is a frequently stated reason for overweight in formula-fed infants. Metabolic and endocrine responses of the body to different milks may be another reason that contributes to increased weight gain in formula-fed infants. A few studies have been conducted recently regarding fuel metabolites and hormone levels in six-day-old formula-fed and breast-fed neonates. These studies show that feeding pattern has an influence on basal and postprandial serum levels of gastrointestinal and pancreatic hormones. The most striking finding was an elevated and prolonged response of serum insulin to formula-feeding. This may be explained by amount and composition of the milk as well as by levels of the hormone gastric inhibitory polypeptide which stimulates insulin secretion. The greater insulin response to formula-feeding may have an effect on weight gain and development of adipose tissue in the infants since insulin is a lipogenic hormone.

A research plan is presented which will investigate the response of several hormones and fuel metabolites to either breast-feeding or formula-feeding. Anthropometric measurements will be taken to provide information about the growth status of infants and the degree of leanness and fatness. Correlating feeding pattern and hormone status to growth development of infants may give some insight to the problems of overweight in infancy and its relation to food intake.