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Regulation of Eicosanoid Pathways: A Pathway to Health and Development

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There are few biological pathways that are more central to overall human and animal health than the eicosanid pathway. The eicosanoids are central not only to the inflammatory pathway, but metabolic diseases such as obesity, arteriosclerosis, autoimmunity, cancer, growth, pain, and development. Tools for the regulation of this pathway have always been with us, but have been neglected and ignored. In this short summary, a description of the eicosanoid pathway, diseases related to the pathway, and a method to manipulate the pathway will be presented. Manipulation of the eicosanoid pathway provides a potential mechanism for the replacement of antibiotics in animal feeds for improving growth and feed efficieny.

Eicosanoid Pathway: Eicosanoids are a family of lipid mediators regulating numerous physiological processes (Hwang, 1989). Theses lipid mediators are derived from the fatty acid, linoleic acid (18:2, cis 9, cis 12), which is an essential nutrient for all animals. Linoleic acid is inserted into the sin 2 position of phospholipids that make up cell membranes. It is modified by desaturase and enlongase enzymes which converts it to arachidonic acid (AA) (20:4). Under the action of numerous stimuli (such as endotoxin, cytokines, hormones, and other cellular stimuli), an enzyme, known as phospholipase A_2, cleaves arachidonic acid from the sin 2 position of phospholipids in the cell membrane. The released AA is converted, depending on cell type, to prostaglandins (PGs) (via cyclooxygenase, or COX) or leukotrienes (LTs) (via lipoxygenase, or LOX). The PGs and LTs signal autocrine, paracrine, and perhaps even endocrine metabolic processes in the host. For example, inhibition of COX is well know to prevent pain, and is the basis for acetylsalicylic acid (aspirin). The discovery of two COX isoforms, COX-1 and COX-2, has shown that basal levels of COX lipid mediators (COX-1 products) are essential to normal health, whereas the products of the COX-2 (which is an inducible enzyme) may have adverse health implications. Recent data on COX-2 knockout mice has shown that many inflammatory processes, such as arthritis, are greatly reduce by specifically inhibiting the COX-2 pathway (Myers *et al*., 2000). Hence, there has been an intensive effort by pharmaceutical companies to design specific drugs that inhibit the COX-2 pathway, without having adverse effects on the constitutive and maintenance pathway of COX-1 (Riendeau *et al*., 2001).

One of the problems of selective inhibition of inducible COX products is that AA cleavage by PLA_2 results in substrate availability for the LOX pathway (Van Wauwe and Goossens, 1983). It is now well recognized that inhibition of COX results in the over production of LOX products (LTs). One of the classical disease situations in which this occurs is aspirin-induced asthma. By the inhibition of the COX pathway, substrate is available for the synthesis of LOX products that are well know to enhance the inflammatory process of the disease. In the case of asthma, an LT antagonist has been developed to inhibit the adverse effects of LTs in the inflammatory process. Hence, regulation of the eicosanoid pathway must consider both COX and LOX regulation.

Eicosanoids in Disease: There is clear evidence that the eicosaniod pathway is involved in many metabolic diseases of animals and humans. Some forms of cancer appear to evade the immune defenses by the over production of prostaglandins, which in turn inhibit immune defense against malignant cell development (PGs are well known immune suppressants) (Marnett, 1992; Thun *et al*., 1993; Hansen Petrik *et al*., 2000; Liu *et al*., 2002).The role of PGs in cardiovascular disease is best shown by the benefits of the use of COX inhibitors. Central in the pathway of fat accretion and metabolism are both the leukotrienes and prostaglandins (Forman *et al*., 1995; Sessler and Ntambi, 1998). Inhibition of COX products is known to prolong the life and improve the quality of life of patients with arthritic and lupus autoimmune disorders (Yang *et al*., 2000), and there is limited evidence that inhibition of the general eicosanoid pathway may improve animal growth and feed efficiency.

If the ecosaniod pathway is important in the regulation of normal physiological processes, what are the consequences of regulating it, and how should it be regulated? Is there the possibility that modern animal and human conditions have put abnormal pressure on the eicosanoid pathway? If one were to regulate the eicosanoid pathway, how would they do it and what would be the unintended consequences and benefits?

A great deal can be written about the consequence and benefit of this pathway. A few comments will be made with regard to select animal health issues.

There are considerable cost in maintaining the interface between the microbial world and the animal. To put it simply, the microbial/animal interface cost, in an animal production context, over $1 billion in production per year, in the absence of overt disease (Cook, 2001; Lev and Forbes, 1959). This loss is mainly in the form of reduced growth and poorer feed efficiency. The loss of productivity (growth and feed efficiency) is not due to the microbial world, but due to the animals' immune defense against the microbial interface. During an immune challenge, the immune cells release a plethora of cytokines, two most notably, Interleukine 1 (IL-1) and tumor necrosis factor (TNF), which directly causes decreased weight gain and decreased feed efficiency (Cook and Pariza, 1998). The adverse effects are due to the eicosanoid pathway (Cook *et al*., 1993). Cytokines such as IL-1 and TNF stimulate cells to produce lipid mediators (eicosanids) that in turn cause the collateral damage associated with the immune reaction. The consequence of immune stimulation is, hence, decreased growth, weight loss, and decreased appetite (cachexia). The eicosanoid products are well known to suppress immune function (Cook *et al*., 1993). The success of aberrant tumor cells, in some cases, is via the over production of select lipid mediators. By the over production of select prostaglandins, tumor cells can suppress immunological rejection of the mutated tissues and thus thrive in the host.

On occasion, the immune system turns against the host. As with any defense system, the host is not always protected from the collateral damage of its defense. One of the major vehicles of collateral damage during autoimmunity are lipid mediators, or eicosaniods. For example, during the autoimmine disease, lupus, where the immune system begins to make antibodies against ones own DNA, immune complexes form that filtrate on the basement membrane of the kidney. Complement is activated, inflammatory eicosanoids are released, attracting inflammatory white blood cells, which in an attempt to eliminate the stimulant, cause damage to the basement membrane. The basement membrane becomes damaged and begins to leak plasma protein in the urine (proteinuria) and the animal eventually dies from kidney failure.

There is evidence that the eicosanoid pathway plays an intimate role in growth and development. This link has been discussed in a number of reviews (Cook, 2001). Briefly, select cytokines from the macrophage cell lineage, induce muscle wasting via prostaglandins, during immune stimulation. The result of eicosasnoid-induced

weight loss is evidenced by reduced performance post vaccination, where decreases in performance are directly linked to cytokine production during immune stimulation. Hence, if one could decrease eicosanoid release during the immune response, one should increase animal growth and decrease the age to sexual maturity.

Regulation of the Eicosaniod pathway as an alternative to antibiotics

Conjugated Linoleic Acid (CLA): In order for a product to successfully prevent the adverse health effects of the eicosanoid pathway, ideally it must allow for basal production of prostaglandins and leukotrienes for house keeping purposes, inhibit inducible eicosanoids, particularly during the inflammatory process, and inhibit both the lipoxygenase and cyclooxygenase pathway in a similar manner, since the inhibition of only one pathway results increased substrate flow to the other pathway.

Since linoleic acid (18:2, cis 9, cis 12) is the precursor for both the LOX and COX enzymatic pathways, we believed that an analogue of linoleic acid would be potentially beneficial in the regulation of eicosanoids. CLA is also an 18-carbon fatty acid with two double bonds. Unlike linoleic acid, the double bonds in CLA are in a geometrical and positional configuration that is unlike linoleic acid. The two major isomers of CLA are cis 9, trans 11, and trans 10, cis 12. Hence, either the 12 carbon double bond of linoleic acid is move to position 11 on the carbon chain in a trans geometry, or the 9 carbon double bond is move to carbon 10, also in a trans geometry. These subtle changes in the precursor (linoleic acid) for eicosanoid biosynthesis were found to have dramatic effects on eicosanoid biosynthesis, and animal health and disease (See www.wisc.edu/cook for CLA bibliography).

CLA was shown to have potent inhibitory effect on antigen-induced eicosanoid release from several smooth muscles without inhibiting basal eicosanoid production (Whigam *et al.*, 2001; Whigham *et al*, 2002). Both leukotrienes and prostaglandins were similar in trachea, bladder and lung from antigen sensitized guinea pigs fed either a control or CLA supplemented diet prior to tissue challenge with antigen. Following antigen challenge of the tissues, ex vivo, LTs and PGs increased in tissues from control fed animals, but release was inhibited in CLA fed animal.

The regulation of eicosanoid biosynthesis is believed to be the central pathway by which CLA has its effects on a wide range of biological activities. CLA was shown to reduce lipopolysaccharide and autoimmune-induced cachexia (Cook *et al.*, 1993; Miller *et al.*, 1994; Yang *et al.*, 2000). CLA was also shown to be a potent inhibitor of cancer of the prostate (Visonneau *et al.*, 1997), colon (Liew *et al.*, 1995), breast (Ip *et al.*, 1991, 1994, 1995) as well as cancer in several other tissues (Pariza *et al.*, 1999). CLA was shown to extend the longevity of the lupus autoimmune mouse model 1.5 to 1.7 fold (Yang *et al.*, 2000), and enhance immune function (Cook *et al.*, 1993; Miller *et al.*, 1994: Sugano *et al.*, 1998). Several unexpected discoveries were that CLA enhanced animal growth and improved feed efficiency (Chin *et al.*, 1994), reduced the clinical signs of arteriosclerosis (Lee *et al.*, 1994; Nicolosi *et al.*, 1997), and reduced body fat (Park *et al.*, 1997). Hence, in some animal species, CLA is a possible candidate for antibiotic replacement with added agronomical and health benefits.

Anti- PLA_2: Another strategy we had to enhance growth and improve feed efficiency in growing livestock was to develop a method to inhibit the enzyme Phospholipase A_2 (PLA_2), which is responsible for the release of substrate for LOX and COX enzymatic pathways. As previously mentioned, linoleic acid is elongated and desaturated into arachidonic acid in the sin 2 position of phospholipid. During select immune stimulation, PLA_2 cleaves AA from phospholipid, where it is in turn converted to LTs and PGs by their respective enzymes (Balsinde *et al.*, 1999). PLA_2 is also an enzyme that bacteria produce as an invasive factor that allows bacteria to gain entry through the intestinal mucosal interface.

The strategy employed was based on some prior work involving the synthesis of egg yolk antibody. We had previously shown that antibody to a neuropeptide, cholecystokinin, was effective in improving animal growth and feed efficiency, and could be used as a replacement for antibiotics (Cook and Jerome, 1998; Cook *et al.*, 1998a; 1998b; 1999). The laying hen is very efficient in transferring antibodies into egg yolk, and was hence immunized with PLA_2. Egg yolks were collected as a source of antibody, dried, and fed to chicks. In a number of trials we found that chicks fed the antibodies to PLA_2 had over a 5% improvement in growth and feed efficiency when compared to control fed chicks (Cook, 2001).

Conclusion: The regulation of inflammatory eicosanoids, both of the lipoxygenase and cyclooxygenase pathways, represents a possible mechanism by which animal growth and feed efficiency can be improved. Unlike antibiotics which target the microbial ecosystem, which induces immune stimulation, and hence the inflammatory response, targets involving how the host responds to the inflammatory response provide an alternative to antibiotics for the purpose of improved growth and feed efficiency. These new targets should focus on providing a buffer between the host and immunological reactivity. By protecting the host from the negative consequence of the immune response (Cook, 1999), we can maintain enhanced productivity without a concern of creating resistant organism, and maintain the integrity of the animal's immune defense. Regulation of the eicosanoid pathway is one mechanism by which this can be accomplished.

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Effect of Heating on Apparent Digestibility of Some Infant Formulations and Cereal-Legume Blends Available in Bangladesh

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Abstract: The apparent digestibility index (DI) of infant formulations obtained from the local market of Bangladesh were studied by monitoring the change in absorbance at 280 nm during enzyme action. Acetone powder of the samples were used as substrate and the enzyme was pepsin (EC.3.4.23.1). In every case, the enzyme protein ratio was 1:12.5. The highest DI was for a milk powder based product called "Product 103" and the least DI was observed for a wheat and fruit based "Product 102" having a DI of 7.57×10^{-4} and 2.29×10^{-4}, respectively. There were no significant differences in the apparent digestibility of most of the infant formulations. Effect of heat treatment on digestibility was assessed after heating for 5 minutes at 100°C. There were significant differences in DI before and after heating. The DI of wheat and fruit based product, "Product 103" was 5.63 fold greater(12.91×10^{-4}) greater than the value before heating.

Key words: Digestibility, *in vitro*, protein, pepsin

Introduction

Protein is one of the five basic components in an adequate diet (Lehninger, 1982). Protein undernutrition is common for millions of people of underdeveloped countries, Bangladesh is one such example. Children of growing age are the worst sufferers. They suffer from many nutritional diseases due to lack of adequate quantity of good quality protein (Lehninger, 1982). On the other hand, it is found that a number of weaning foods are marketed in the local market of Bangladesh.

The nutritive values of dietary protein depends primarily upon the concentration and distribution pattern of their constituent amino acids (Bodwell *et al.*, 1980). Amino acid composition data thus generally indicate the protein nutritive value of various protein sources. However, nutritive value as estimated by animal assays is often lower than that predicted from amino acid data. This is attributed, in part, to a lack of complete availability of all of the amino acids due to incomplete digestion of the protein.

In vitro methods of protein evaluation are useful in screening new protein foods and processing methods because of their rapidity (Dimes and Haard, 1994; Walter and Mark, 1964). Reviews of laboratory methods of protein quality evaluation have been reported by Swaisgood and Catignani (1982). An *in vitro* digestion is a convenient and rapid way to assess the potential bioavailability of proteins by enzymatic digestion under model conditions. Food proteins are digested by proteolytic enzymes. These enzymes catalyze the hydrolysis of specific peptide bonds (Cheftal and Cug, 1985; Fennema, 1985). Numerous methods have been used to mimic the *in vitro* digestion of proteins (Madisetty, 1991).

The objective of the present study was to assess the *in vitro* digestibility of commercial infant formulations commonly available in Bangladesh before and after heat treatment. Enzymatic proteolysis was used as the tool to quantitate digestibility.

Materials and Methods

Infant formulations (Products 101-108)(Table 1) and fresh wheat and chickpea were obtained from the local market of Bangladesh. Blended cereal-legume mixes comprising of roasted wheat and chickpea, with (Complete Blended Food) and without vitamin/mineral mixture (Blended Food)(Table 1), were donated by the infant food production facilitiy of Ganashastha Kendra at Teknaf, Bangladesh. Sodium phosphate dibasic, sodium phosphate monobasic and diethyl ether were obtained from Aldrich Chemical Company, Inc., Milwaukee, WI; glycine and acetone were obtained from Fisher Chemicals, Fisher Scientific; The reference food protein bovine serum albumin(BSA), ovalbumin (OVA, and pepsin (EC.3.4.23.1) were obtained from Sigma Chemicals Company, St. Louis, MO. All other reagents were analytic grade.

Buffers: Extraction buffer (33 mM phosphate buffer pH 6.8). The extraction buffer that was used for solubilizing the infant formulations was 33 mM sodium phosphate at pH 6.8.
Digestion buffer used for the digestion experiment was 0.2 M glycine-HCl at pH 2.6.

Preparation of acetone powder: Acetone powder of the samples were obtained by an improvement of the method as described by Haque and Kito (1982). Three grams of food sample was dissolved in 20 ml of extraction buffer (33 mM phosphate buffer pH 6.8) and mixed thoroughly with shaking. The tube was then centrifuged at 4000 RPM (3077 x g) for 10 minutes in a TECHNOSPIN R (DuPont) centrifuge . After centrifugation, the pellet was discarded, supernatant was collected and kept in a salt-ice bath of -2°C. Acetone (1 fold v/v) chilled to -20°C overnight was added to the cooled supernatant. The supernatant was allowed to stand for 45 minutes in the salt-ice bath in order to get good precipitation. The sample was centrifuged at 4000 RPM (3077 X g) for 15 minutes. The supernatant was discarded by decanting, and the protein precipitate was collected. An equal volume of diethyl ether (ice cold) was added to the precipitate to wash away residual lipids. Protein precipitate thus collected was thoroughly dried of solvent under a stream of air, redispersed with distilled water, and lyophilized in a LABCONCO (Labconco Corp., Kansas City, MO) freeze dryer system. Each acetone powder was stored in sealed containers and kept at -10°C until needed.

Preparation of heat treated samples: Heat treatment of the infant formulations and food samples were carried out at 100°C in a thermostated water bath (Isotemp Refrigerated Circulator, Model 90, Fisher Scientific) for 5 minutes. The samples were then transferred to an ice bath (at 4°C) for another 5 minutes. After that, the samples were equilibrated to room temperature for further experiment.

Determination of apparent *in vitro* digestibility: *In vitro* digestibility was assessed by the method described by Haque and

Kinsella (1988) and Haque and Khalifa (1992). Enzyme to protein ratio was kept constant at 1:12.5 for all experiments. A freshly prepared pepsin solution (25 μL) was injected into a temperature equilibrated (37°C) micro-cuvette containing 500 μL of acetone powder solution in digestion buffer. A high precision HPLC grade syringe (Hamilton Co, Reno, NV) was used for injecting the enzyme solution. Temperature was controlled by a water-jacketed micro-cuvette holder connected to a temperature controlled water bath. A micro-stirrer (Instech Labs Precision Controller, Plymouth Meeting, PA) was used to stir the content of the cuvette (the acetone powder solution and enzyme) constantly at a minimum attenuation using a Teflon probe. Care was taken to ensure that the stirrer was not in the path of the light beam. Change in absorbance at 280 nm was recorded every 15 sec. after the enzyme was injected into the acetone powder solution. A computer-assisted (SpecScan Software) Spectronic 1201 Milton Roy Dual Beam Spectrophotometer was used for all experiments. The spectrophotometer electronically compensated for possible instrument drift with time (Haque and Khalifa, 1990). Results are the mean values of three separate determinations.

Calculation of apparent digestibility: The curve was plotted taking absorbance (X-axis) and time (Y-axis) (Fig.1).
Absorbance values were obtained from the computerized printout of data obtained from Spectronic 1201 Spectrophotometer. The absorbance value for each sample was plotted every 0, 3, 6, 9, 12, and 15 min. Then the slope of the curve was calculated. The slope was used as the index of digestibility. Each value was mean of three separate determinations.

Results

Roasted milled chickpea had the higher digestibility (87.80X 10^{-4}) (Table 1). Raw chickpea followed roasted milled chickpea with a digestibility index of 62.93 X 10^{-4}. The least digestible reference food was BSA.
Among the reference foods, it was observed that roasted wheat was more digestible compared to raw wheat (Table 1). This may be due to conformational change of proteins during roasting which made it more susceptible to digest. This is also true for roasted milled chickpea, it was even more digestible than reference protein, OVA and BSA.
The most digestible infant formula was "Product 103" having a digestibility index of 7.57 X 10^{-4} (Table 1). The weaning food composed of wheat and fruit named "Product 102" was the least digestible in our study, with a DI of only 2.29 X 10^{-4}.
The infant formulations studied here are milk-based weaning foods of different combinations. It was found that *in vitro* digestibility did not vary significantly. Product 106, Product 108, and Product 107 are manufactured by the same manufacturers and they ranked 11th, 12th, and 14th, respectively. The decreaed digestibility of " Product 107" may be due to the wheat proteins. There was significant difference between digestibility of food samples before and after heating (Table 1, Fig. 2). Product 102 showed highest percentage of change in DI after heat treatment. It increased to 12.91 from 2.29 reflecting a 463% change. "Product 103" showed the least percentage change in DI before and after heating (Table 1).
Product 103, product 101, and product 105 are infant formulations that are not combined with other plant proteins. These samples had DI of 7.57 X 10^{-4}, 5.10 x 10^{-4}, and 3.97 X 10^{-4}, respectively (Table 1). They ranked 5th, 8th, and 10th. Product 104 had a much lower digestibility index compared to these samples. It ranked at 13th with a slope value of 2.80 X 10^{-4} (Table 1).

Discussion

In vitro digestibility is a useful method for protein quality evaluation (Walter and Mark, 1964). There were two procedures widely used for screening potential protein-food stuffs based on the total amino acid composition - chemical score and essential amino acid index (Swaisgood and Catignani, 1982). But nutritive value of proteins depends on the complete availability of all the amino acids, so a complete digestion of a protein is necessary. In our study, we determined the *in vitro* digestibility of infant formulations as a measure of protein quality.
In vitro digestibility of protein can be estimated by various means (Madisetty, 1991). Our method (Haque and Kinsella, 1988) was rapid compared to the method described by Walter and Mark (1964) and later by Alaknani *et al.*, 1994. This is a spectrophotometric method where the change in absorbance were monitored during enzyme action.
In an experiment, (Alaknani *et al.*, 1994) showed the comparison between different digestion procedures for the multi-elemental analysis of human milk and a representative variety of infant formulas. The effects of digestion procedures and the mass of reference samples on the recovery, precision, and accuracy of multi-elemental analysis were examined. The digestibility was determined by using 0.05 g samples digested in 1.0 ml of concentrated HNO_3 on a hot plate set at 70°C for 5 days and measured directly.
Protein and amino acid digestibility and protein quality of liquid concentrate and/or powder forms of infant formulas were studied by rate balance and growth methods (Sarwar *et al.*, 1989). In this experiment, Sarwar *et al.* (1989) did not compare the digestibility

Table 1: Effect of Heat Treatment on Digestibility Index (DI)

Name of Sample	DI Before Heating X 10^{-4}	DI After Heating X 10^{-4}	% Change
Product 101 (Bebelac)	5.10^{ef}	9.00	+76
Product 102 (Dano Infant-Wheat & Fruit)	2.29^{g}	12.91	+463
Product 103 (Dano Milk Powder)	7.57^{d}	7.73	+2
Product 104 (Biomil Milk Formula)	2.80^{fg}	2.33	-16
Product 105 (My Boy Eldorin)	3.97^{efg}	1.49	-62
Product 106 (Nestle Rice)	3.93^{efg}	2.87	-26
Product 107 (Nestle Wheat)	2.52^{g}	4.00	+59
Product 108 (Nestle Lactogen)	3.73^{efg}	3.20	-14
Blended Food	6.00^{de}	11.20	+86
Roasted Milled Chickpea	87.80^{a}	57.30	+34
Raw Chickpea	62.90^{b}	18.50	-70
Complete Blended Food	4.17^{efg}	8.44	+102
Raw Wheat	5.83^{de}	2.23	-61
Roasted Wheat	7.70^{d}	3.61	-53
Ovalbumin (OVA)	14.73^{c}	1.29	-91
Bovine Serum Albumin (BSA)	1.83^{g}	1.33	-27

$^{a, b, c, etc}$Means followed by the same letter are not significantly different at $P<0.05$

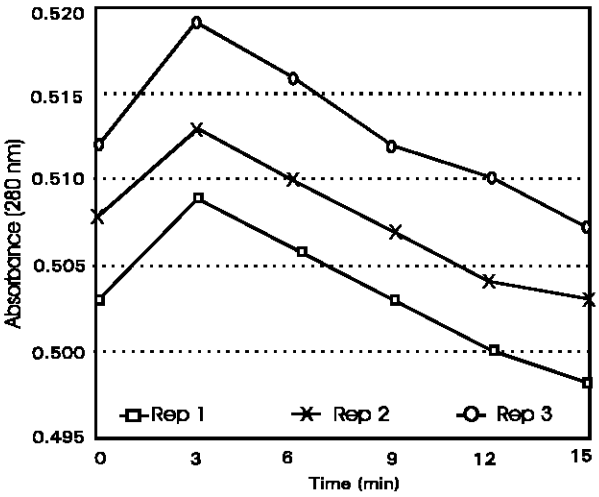

Fig. 1: Absorbance vs Time curve for BEBELAC

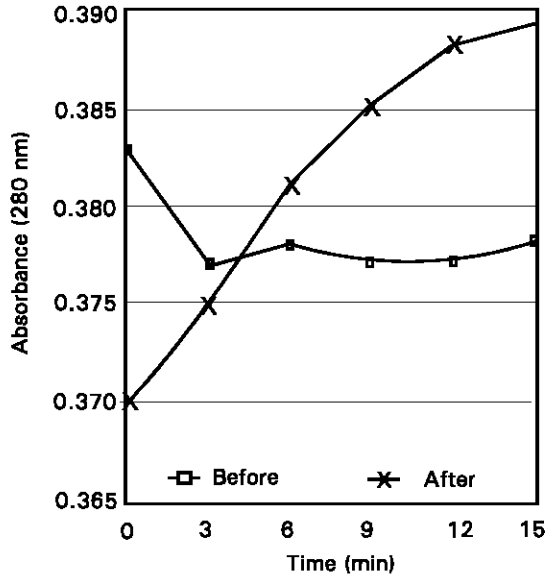

Fig. 2: Effect of heating on digestibility

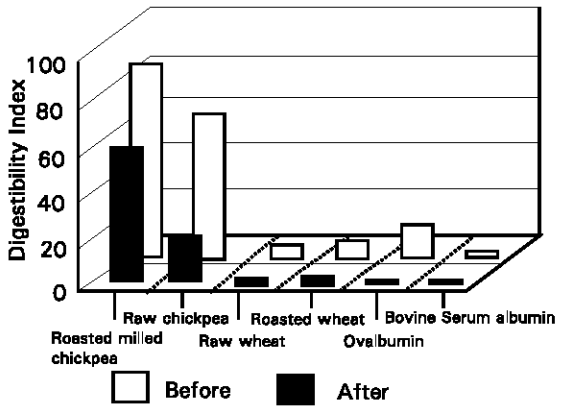

Fig. 3.1: Effect of heat treatment on digestibility index (DI)

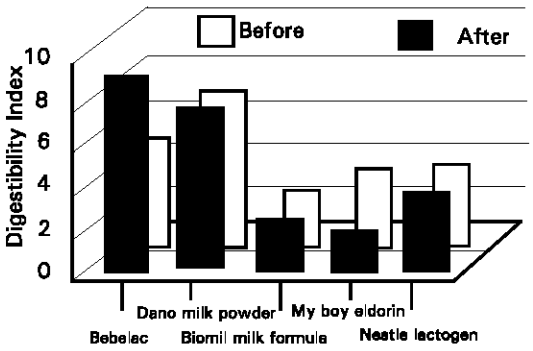

Fig. 3.2: Effect of heat treatment on digestibility index (DI)

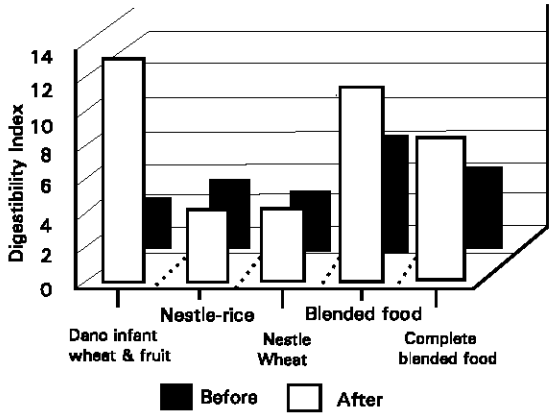

Fig. 3.3: Effect of heat treatment on digestibility index (DI)

of different infant formulations. They compared the difference in protein digestibility of liquid concentrates and powder forms of some different infant formulas. He found that digestibility of protein in liquid concentrates were up to 13% lower than those in powders.

An *in vitro* enzymatic digestion is a convenient and rapid method to assess potential bioavailability of protein under model condition. It was found that protein digestibility were not varied significantly among the infant formulations studied with some exception but there was a significant difference between the DI value of infant formulation before and after heating (Table 1, Fig. 3.1,3.2, and 3.3). Some become more digestible and some less digestible. It was found that legume based infant formulations showed a marked rise in DI after heat treatment (Fig. 3.3). Since all of the infant formulations are prepared from milk based proteins, we can conclude that the difference between digestibility may be due to the presence of non milk based proteins or due to the different types of processing techniques. Detailed *in-vivo* studies are required to establish these observations.

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The Essential Fatty Acids and the Diet of Polar Bears

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Abstract: Plasma lipids of polar bears are significant because these bears prefer to consume high quantities of fat; furthermore one population fasts each year for over four months. In this paper plasma lipids of fed polar bears were compared to fasted bears. Fasted bears were hyperlipidemic to fed bears; both were hyperlipidemic to normal human plasma, in respect to cholesterol and triglycerides. In lipoproteins, the HDL (High Density Lipoproteins) triglyceride was very low as in human subjects in both fed and fasted animals. The other two, LDL (Low Density Lipoproteins) and VLDL (Very Low Density Lipoproteins) were consistently higher in fasted bears than in fed bears, and these fasted bears had much higher cholesterol and triglycerides than the fed bears. Since the fed bears seem to be protected against hyperlipidemia, the fatty acid composition of serum lipids was analyzed. The n-3 fatty acids not the n-6 type dominated in fed bears. These n-3 fatty acids (which were not available to fasted bears) seem to protect against high serum lipids. These results seem to support the concept of using fish oil capsules in the human clinic.

Key words: Plasma lipids, hyperlipidemia, omega-3 fatty acids, polar bear diet, fasted polar bears, lipoproteins of polar bears

Introduction

In human subjects, elevated plasma lipid concentrations appear to predispose to the premature development of atherosclerosis (Lees and Karel, 1990). The approach of comparative physiology elucidates our understanding of this relationship. When plasma lipid is elevated artificially in the pig (Lees and Karel, 1990), the goat (Richard, 1990) and the rabbit (Bolton-Smith *et al.*, 1988), fatty lesions develop in the aorta. It seemed reasonable to us to study a species, which in the free environment, and in our laboratory, selects a diet of nearly 100% fat (Folk, 1996). This species is the polar bear, a hibernator to a limited extent (Folk, 1981). This unusual diet is clearly associated with the fact that the polar bear is one of the most cold tolerant of mammals. By comparison, typical human diets are: Chinese 15% fat, U.S.37%, cold-weather natives vary from 42 to 50% (Story and Weaver, 1990). It is helpful to match the percent of fat in the diets of mammals with their susceptibility to atherosclerosis.

It seemed valuable to study polar bears under two circumstances: (1) when they were selecting nearly pure fat as a diet, and (2) when they had been fasted for at least one month but probably four months. In this way the effect of diet on the plasma lipids of this unusual species could be determined. Another facet of a study of polar bear diet is the fact that the fat selected by polar bears is from seals and whales. This food has a high content of those fatty acids which are called essential (Andersen *et al.*, 1984). The first part of this plan has been completed and published, i.e. a report on two polar bears selecting a diet of nearly 100% fat (Kaduce *et al.*, 1981). The present paper compares the former results with data from two fasted bears.

Materials and Methods

Collection of Plasma: In the previous published study, venous blood samples were obtained from a 450 kg male polar bear, captive for 6 years, and a 205 kg female brought in from the Arctic icepack for the experiment and then released. They were fed dead seals from which they ate the blubber and a small amount of skin.

In the second experiment, venous blood samples were obtained from two polar bears, 176 kg male and a 124 kg female. These two bears were both captured in traps near Churchill, Manitoba, Canada on October 1, 1990. They were initially ice-transported bears because the prevailing wind is south in Hudson Bay. When the ice melted about July 1, they were deposited on the shore. They had probably spent the summer walking up the west shore of Hudson Bay to Churchill. The survey of the Canadian Department of Natural Resources indicated that apparently there was no available food for these bears, such as dead animals.

They were maintained in captivity during the entire month of October, and the blood samples were taken on November 6, 1990. While in captivity, the bears were not fed, were given only water, and were almost totally prevented from even the sight of human beings. Although some polar bears eat kelp and berries while walking to Churchill, their feces can reveal this diet later in captivity (Deroucher *et al.*, 1993). Our two bears had not eaten kelp or berries and had probably fasted the previous three months as well as one month in captivity. The male (#3335) was six years old and had not been trapped before; the female (#9056) was nine years old and had been trapped on two other occasions. She was not lactating and she had given birth to a cub in1981, and another in 1988. After the blood samples were obtained, the two bears were flown by helicopter and released by the Canadian Department of Natural Resources over 100 miles north and beyond Fort Prince of Wales. These bears had been present at Churchill waiting for sea ice to form. This takes place on approximately November 15, although there is a trend now for the ice to form later (Stirling *et al.*, 1999).

The venous samples were obtained while the polar bears were under the influence of the immobilizer Telazol. The procedure was completed in approximately 20 minutes, and there were no convulsions associated with this drug.

The blood was drawn into tubes containing 1 mg of ethylene diamine tetra-acetate (EDTA) per ml of blood and immediately centrifuged for 10 minutes, and then transferred into screw-capped vials. The plasma was transported in a frozen condition to Iowa City. After three days it was thawed for analysis.

Isolation of the Lipoproteins: Lipoproteins were isolated from the blood plasma by ultra centrifugal flotation using the procedure of Havel *et al.* (1955) as modified by Brenneman and Spector (1974). The VLDL* fraction was isolated at a density of 1.006 g/ml, LDL at 1.063 g/ml, and HDL at 1.21 g/ml. The VLDL was washed once, and the LDL and HDL fractions were washed twice by ultra-centrifugal flotation through KBr-NaCl solutions of appropriate densities. The washed lipoprotein fractions, each having a volume of approximately 3 ml, were dialyzed for 72 hours against one liter of 0.154 M NaCl which contained 0.02 % NaN_3. The dialysis was changed three times.

Each of the lipoprotein fractions migrated as a single band on

agarose gel electrophoresis (DHEW publication No. 75-628, 1974).

Lipid Analyses: The triglycerides, cholesterol, cholesterol ester, and phospholipid content were measured in the intact plasma; the three isolated lipoprotein fractions, and the residual plasma of density were greater than 1.21 g/ml. Triglyceride and total cholesterol content were determined using an automated colorimetric and fluorometric analysis (DHEW Publication No. 75-628, 1974). The phospholipid content of the plasma and isolated lipoprotein fractions were estimated from measurements of organic phosphorus. Lipids were extracted from the samples by the method of Folch (1957). Aliquots of the lipid extract contained in the washed $CHCl_3$ phase of the extract were dried under N and assayed for organic phosphorus by the method of Raheja *et al.*(1973). The total and free cholesterol contents of the samples were measured using the method of Searcy and Bergquist (1960).

Fatty Acid Composition of Serum Components: The fatty acid composition of the phospholipids, cholesteryl esters, and triglycerides in the serum was determined using a combination of column and gas liquid chromatography (GLC). Silicic acid columns containing 2g of Unisil (Clarkson Chemical Company, Williamsport, PA) were employed to separate the lipids into classes. The lipid mixture was added to the column in hexane. After the column was washed with hexane, cholesteryl esters were eluted with diethyl ether–hexane (1:50 v/v) (Carroll and Serdarevich, 1967), neutral lipids were eluted with chloroform, and the phospholipids were eluted with methanol (Christie, 1973). The fatty acids contained in each of the lipid classes were hydrolyzed by saponification with 5 ml of 95% ethanol and 0.12 ml of the 33% KOH for 45 minutes at 70° C. Nonsaponifiable material was removed by extraction into n-hexane. The aqueous phase was acidified, and the fatty acids then were extracted into n-hexane. Following removal of the organic solvent by evaporation under N_2, methylation of the fatty acids was carried out for 10 minutes at 95° C using 14% anhydrous boron trifluoride in methanol (Applied Science Inc., State College, PA). Fatty acid methyl esters then were extracted from the reaction mixture with three 3ml portions of n-hexane. The hexane was evaporated under N, and the fatty acid methyl esters were taken up in 25 l of carbon disulfide and separated by gas-liquid chromatography. Glass columns, 4mm x 6ft packed with 10% Apolar 10 C on gas Chrom Q (Applied Science, Inc. State College, PA) and a Hewlett-Packard model 5710A gas chromatograph equipped with a flame ionization detector were employed to separate and quantify the methyl esters. Peak areas were measured using an Infotronics model CRS 100 digital integrator. Peaks were identified by comparing their retention times with those of known fatty acid methyl ester standards (Supelco Inc., Belefonte, PA, and Nu-Check Prep, Elysian, NM).

Results

All results from the fasted animals will be compared with determinations from the male and female polar bears that had previously been maintained for several months on a fat diet. The results from fed bears have been published, but some of them are included to be compared in this paper. In all tables, the description "fasted" applies to the two bears that probably did not eat for four months.

Lipid Composition of Serum: Both the male and female fasted bears had approximately the same concentration of triglycerides but a larger difference in cholesterol (fasted male 333 mg/dl; fasted female 429 mg/dl). This same difference between sexes was found in the animals that had been eating fat.

When the sexes were combined, the cholesterol of the fed bears (mean 298 mg/dl) and the fasted animals (mean 381 mg/dl) represented values that were so high that in the human clinic, they would be considered seriously pathological (Table 1). The free/total cholesterol ratio was very similar in both the fed and the fasted animals.

Table 1: Plasma Lipid Analyses of Fed and Fasted Polar Bears

	Fed (mg/dl)	Fasted (mg/dl)
	Avg ± Standard Error of the Determination (n=3)	
Cholesterol	298 ± 24	381 ± 36
Triglycerides	199 ± 15	292 ± 7

Table 2: A Comparison of Plasma Lipid in Greenland and Danish Residents (From Bang *et al.*, 1971)

	Greenland Inuits (mg/dl)	Danes (mg/dl)
	n=61♂	n=61♂
Cholesterol	233	273*
Triglycerides	57	129*

*P>0.05

Table 3: Polar Bear Plasma Phosholipid Fatty Acid Composition

Fatty Acid	Fed	Fasted
	% Composition (Avg ± Standard Error of the Determination. n=3)	
<16:0	0.6±0.1	1.5±0.1
16:0	11.6±0.3	22.4±4.7
18:0	37.7±0.8	35.0±0.5
16:1	5.5±0.4	2.2±0.6
18:1	27.2±0.1	11.9±2.4
18:2n-6	2.5±0.2	6.7±1.1
18:3n-3	3.4±0.2	0.5±0.2
20:4n-6	3.4±0.1	9.1±0.1
20:5n-3	4.4±0.4	3.5±1.4
22:5n-3	0.8±0.3	0.7±0.3
22:6n-3	2.5±0.4	2.0±1.0
Others	2.5±0.4	3.2±0.2
	Fatty Acid Classes (Avg. of the % Distribution)	
Saturates	49.9	57.4
Monounsaturates	32.7	14.0
Polyunsaturates	14.9	23.9
n-6 Polyunsaturates	5.9	15.8
n-3 Polyunsaturates	9.0	6.2

Our hypothesis had been that the fasted bears would have lower lipid values than the fed bears. Note instead that the values for fasted bears were relatively high (Table 1). It was possible that some material in their diet was protecting the "fed" bears. We looked for guidance in testing this idea by turning to another case of a high fat diet; this example involves human subjects. Bang and coworkers studied Greenland Inuit eating a 50% fat diet of sea mammals and cold-water fish. They too seemed to be "protected" from high plasma lipid by their diet that was similar to our fat-eating polar bears (Table 2). A detailed analysis of the fatty acid (FA) composition of the polar bear serum lipids might give a clue to what this "protection" is.

Fatty Acid Composition of Serum Lipids: Results of the gas-lipid chromatographic analysis of the fatty acids in the plasma lipids are shown in Table 3, 4, and 5. In the fasted male and female, most of the total fatty acids contained in the phospholipids were either saturated (57.4%) or polyunsaturated fatty acids (PUFA). Only 14% were monoenoic. The trend was different in the triglycerides: 36% saturated FA and 29% monoenoic FA. In the cholesterol esters there were even lower saturated FA (25%) with 33% monoenoic.

Some specific selected triglyceride and cholesterol ester fractions of fasted bears contained high amounts of saturated acids, for example 27% (16:0) and 21% (16:0) respectively. However, the most striking results for these fasted bears were in the n-6 and n-3 series: for triglycerides, the combined percents were 14% (n-6)

Table 4: Polar Bear Plasma Triglyceride Fatty Acid Composition

Fatty Acid	Fed	Fasted
% Composition (Avg ± Standard Error of the Determination. n=3)		
<16:0	0.6±0.1	0.5±0.5
16:0	6.4±0.4	26.6±9.8
18:0	4.7±0.6	9.4±1.9
16:1	10.9±1.6	8.1±2.3
18:1	33.8±2.8	20.9±2.6
18:2n-6	3.1±0.4	9.3±0.2
18:3n-3	4.6±1.0	0.6±0.2
20:4n-6	2.3±0.1	6.0±1.4
20:5n-3	12.7±0.9	3.8±1.9
22:5n-3	3.6±0.4	1.1±0.1
22:6n-3	13.7±3.2	6.0±0.7
Others	3.1±0.6	4.5±2.4
Fatty Acid Classes (Avg. of the % Distribution)		
Saturates	12.3	35.9
Monounsaturates	44.7	29.1
Polyunsaturates	40.0	25.9
n-6 Polyunsaturates	5.4	14.3
n-3 Polyunsaturates	34.6	10.9

Table 5: Polar Bear Plasma Cholesterol Ester FaM Acid Composition

Fatty acid	Fed	Fasted
% Composition (Avg ± Standard Error of the Determination. n=3)		
<16:0	1.4±0.1	2.1±0.9
16:0	7.8±0.2	21.4±0.7
18:0	1.6±0.2	3.8±0.9
16:1	14.6±0.2	8.5±2.6
18:1	25.7±0.9	24.1±2.2
18:2n-6	5.0±0.4	20.8±3.7
18:3n-3	0.9±0.2	0.3±0.1
20:4n-6	11.4±0.7	9.7±1.0
20:5n-3	29.3±1.4	3.2±0.1
22:5n-3	0.4±0.3	0.7±0.2
22:6n-3	0.8±0.1	3.0±0.5
Others	1.0±0.2	1.8±0.3
Fatty Acid Classes (Avg of the % Distribution)		
Saturates	10.8	25.2
Monounsaturates	40.3	32.6
Polyunsaturates	47.8	38.3
n-6 Polyunsaturates	16.4	30.5
n-3 Polyunsaturates	31.4	6.8

and 11% (n-3) and for cholesterol ester, 31 and 7%. The high value for n-6 is especially noticeable when considering the relative importance of the n-6 and n-3 FAs in this outdoor situation.

Now comparing fed and fasted bears, the phospholipid values were nearly the same in most cases (fed 396 mg/dl, fasted 395 mg/dl). Concerning triglycerides, saturated fatty acids (16:0 and 18:0) were very much higher in fasted bears; this finding was reversed for nearly all unsaturated fatty acids. There are eight specific selected FA's to consider (Table 4). In six of these cases there was a much larger percent in the serum lipid of the fed compared to fasted bears. Comparing the cholesterol ester figures, saturated fatty acids (16:0 and 18:0) in the fasted bears were up to three times the amount of saturated acid compared to the fed bears. Comparing unsaturated examples, there were eight cases of pairs to be assessed in Table 5. In five cases the differences were in favor of the fed animals.

Looking at the FAs as a whole, the n-6 FAs were not conspicuously larger in the fed animals; the large differences were found in the n-3 series. For example, in cholesterol ester (Table 4) the 20:5 n-3 was 29.3% in the fed animals and 3.2% in the fasted. Summarizing triglycerides (Table 4), total n-3 values were 34.6% fed, compared with 10.9% fasted; for cholesterol ester (Table 5) the total n-3 values were 31.4% fed and 6.8% fasted.

Lipid Analyses of Lipoprotein Fractions: The lipoproteins of all classes were higher in most cases in the fasted bears than fed bears (Table 6). An analysis of the phospholipid alone in the lipoproteins of all bears showed that the majority was contained in the HDL fraction and as expected a small amount in LDL and VLDL. This picture was the same for total cholesterol: there was a substantial amount in HDL, a somewhat larger amount in LDL, and the expected low value of VLDL. The triglycerides for the fasted bears showed a reverse relationship with very small fractions of HDL, much larger fractions of LDL compared to the phospholipids, and an unusually large figure of 49 mg/dl for VLDL. We now consider the ratio between HDL and LDL for fasted bears only. Based upon information from human subjects, we can call the ratio for the phospholipids a healthy one (421 mg/dl HDL vs. 92 mg/dl). This ratio was reversed as expected in the triglycerides (16 mg/dl vs. 145 mg/dl). The expected ratio was not found in total cholesterol, 165 mg/dl HDL vs.194 mg/dl.

Of greater interest is the comparison of the lipoproteins in fed bears compared with fasted. Once again we are looking for a possible protection which benefits the bears eating 100% fat. First consider the VLDL. It is lower in all cases in fed bears; in our later discussions this will be considered an advantage for fed bears.

Next, consider the HDL. There is no trend favoring the fed bears except in cholesterol; the benefit seems to be in favor of the fasted bears with their higher HDL in phospholipids and triglycerides.

Of lesser importance are the LDL determinations; the three relatively low values (70, 121, 51 mg/dl) for fed bears are important in later discussion.

The final analysis compares fed bears with fed human subjects (Table 7). The low VLDL figure for fed bears is 9 ± 2 mg/dl, consistent with healthy human subjects (Pruzanski *et al*, 2000). The HDL figure of 170 ± 6 for the bears is extraordinarily high.

Discussion

Why do polar bears eat high fat?: What is the species' advantage of eating nearly 100% fat? Polar bears must do this because of their habitat that consists of the ice pack of the Arctic region. They often are forced to tolerate a long fast sometimes in continuous darkness that may last over 90 days. Perhaps eating 100% fat is the most efficient way to lay down the necessary abundant depot fat when food is available. The longest fast known of any mammal (8 mos.) is found in the female polar bear of Hudson Bay.

Result of high fat diet: The mean cholesterol reported in this paper range from 306 mg/dl to 381 mg/dl. Such high values would be fatal to the domestic dog and rabbit (Ferguson and Folk, 1971). Note that the first of these values was obtained from the bears eating nearly 100% fat while the second value was obtained while the bears were living on abundant depot fat. In an earlier article, we had published high values of cholesterol and triglycerides from polar bears, but the samples were obtained from running bears immobilized from a helicopter. The values in mg/dl were: cholesterol 370, 257, and 353; the results for triglycerides were 249, 352, and 342 (Nelson *et al.*, 1983). The results are consistent with our measurements from captive bears.

How does the bear tolerate high cholesterol?: This experiment was designed with two fasted bears to try to determine how they can tolerate high cholesterol. The fasted bears had considerably higher cholesterol esters in the plasma than the bears eating high fat. In Results, we reported high saturated fatty acids (16:0 and 18:0) in both cholesterol and triglycerides of the fasted bears. Since saturated FAs would tend to raise both substances in serum (Huang and Nassar, 1990), their presence helps to explain the high values of cholesterol and triglycerides in the fasted bears. When

Table 6: Composition of Polar Bear Lipoproteins

	VLDL		LDL		HDL	
	Fed	Fasted	Fed	Fasted	Fed	Fasted
	(mg/dl)					
Phospholipid	7 ± 1	10 ± 3	70 ± 5	92 ± 12	308 ± 19	421 ± 15
Triglycerides	30 ± 7	49 ± 10	167 ± 13	145 ± 22	5 ± 1	16 ± 6
Cholesterol						
Total	9 ± 2	27 ± 12	121 ± 19	194 ± 30	170 ± 6	165 ± 18
Ester	0 ± 0	2 ± 1	51 ± 3	77 ± 3	78 ± 1	70 ± 2

Avg. ± Standard Error of the Determination (n = 3)

Table 7: Human and Fed-Bear Plasma Lipids

	Normal Human*	Fed Polar Bears#
	(mg/dl)	
Triglycerides	35-160	199 ± 15
Total Cholesterol	< 200	306 + 22
VLDL	6-40	9 ± 2
LDL	< 130	121 ± 9
HDL	> 35	170 ± 6

* (Havel *et al.*, 1955)

\# Avg ± Standard Error of the Determination (n = 3)

we examine the eight specific selected unsaturated fatty acids in pairs (fed vs. fasted) we find that the n-3 series was much higher in the fed bears relative to the fasted bears; however, the n-6 fed values were not high.

It is reasonable to suppose that the high level of n-3 fatty acids protected the bears eating high fat. Other species have been protected by a high level of these n-3 fatty acids (Lees and Karel, 1990; Ando *et al.*, 2000; Angerer and von Schacky, 2000) as well. Our fed bears ate seal blubber and some cold-water fish. The most abundant fatty acid in these two foods is the n-3 type (Kuhnlein, 1993, Huang and Nassar, 1990). No mammals can synthesize the essential fatty acids n-3 and n-6 (Uauy and Valenzuela, 2000). They are synthesized by phytoplankton in the ocean that is eaten by small crustacea (krill) which are eaten by fish and whales. Seals eat the fish. The polar bears are at the top of the food chain and eat both seal and whale. Thus the protective material of n-3 fatty acids reaches the polar bear as well as human subjects in their diet.

One might ask how does the polar bear eating mostly blubber obtain essential amino acids. We have observed our polar bears to eat a small amount of skin which would provide some of this necessary ingredient, and apparently blubber contains 3% protein. The most important point, however, is that relative to the controls (the fasted polar bears) the bears eating high fat had much lower cholesterol and triglycerides. We must presume that this lowering is under the action of the n-3 fatty acids which were not in the diet of the fasted bears..

How do cold-weather natives tolerate high cholesterol?: Greenland natives live with low cholesterol and triglycerides (Table 2). These natives were eating a 50% fat diet. Since they were eating a diet similar to that of our fed polar bears, it must be presumed that they also were protected by n-3 fatty acids (von Schacky, 2000). We estimate that there are some 90,000 cold-weather natives of the Inuit type; undoubtedly more than half of these live on native foods especially sea mammals and cold-water fish and therefore are obtaining n-3 fatty acids in their diet (Aubrey, 1990; Caulfield, 1993; Kuhnlein, 1993; Malcom, 1993). The n-3 quantity in the adipose tissue of Greenland natives has been analyzed (Boudreau, 1993) and was found to be over double the amount in the adipose tissue of Alaska natives and triple the amount in non-native Alaska residents.

Do the lipoproteins also protect fed bears?: The HDL values for total cholesterol were higher in the fed bears compared to the fasted bears. Also for all determinations, the ratio between HDL and LDL were favorable for both fed and fasted polar bears. The lipid content of the isolated lipoproteins was high compared to human readings. However, since the total lipid concentrations in all bears were unusually high, one would expect the lipoproteins also to be high. As pointed out in Results, this was the case (HDL was 170 mg/dl, Table 7).

The total lipid concentrations in fasted animals were highest; and as expected, isolated lipoproteins are highest. The HDL in both fed and fasted animals were much higher than both VLDL and LDL in phospholipids and total cholesterol as in healthy human subjects. The HDL triglyceride was very low as in human subjects. The bears eating n-3 fatty acids (fed bears) tended to have lower values of lipoprotein lipids than fasted bears that were eating no n-3 fatty acids.

VLDL was usually much lower than the other lipoproteins regardless of diet. The n-3 fatty acids appear to raise HDL cholesterol to an unusually high level. VLDL is relatively low in polar bears compared to human subjects. LDL, based on cholesterol content, in fed bears is similar to human, but fasted bears are high compared to human subjects. HDL-cholesterol is very high as if to protect, compared to human subjects. The lipoprotein lipid content of fasted bears was much higher (with two exceptions) than fed bears. A plausible explanation is the presence of n-3 FA in the diet of fed bears eating nearly 100% fat; these FA's are very low in fasted bears.

What is the effect of "protection" on cardiovascular disease?: The habitual diet of polar bears contains a high percent of fat. Do they show a high incidence of cardiovascular disease? We surveyed the records of the Armed Forces Institute of Pathology and of the necropsies in three zoos and in two wildlife national service organizations. There is absolutely no record of any cardiovascular disease in polar bears, some of which had been maintained for as long as 33 years in captivity. One of our polar bears that had levels of 180 mg/dl triglycerides and 270 mg/dl cholesterol was studied until he reached the age of 21 years. At that time after many years of being on a high fat diet from sea mammals and marine fish, at necropsy this animal had no cardiovascular disease or atherosclerosis.

Since polar bears habitually eat a diet high in n-3 FAs it would seem that they are protected from cardiovascular disease. Does this protection also apply to the large population of cold-weather natives who habitually live on a preponderance of sea mammals and occasionally caribou? According to Caulfield (1993) the Greenland Inuit as a whole eat no more than 16% imported store-bought food. A recent survey by Mulvad *et al.* (1996) showed an extremely low rate of ischaemic heart disease in Greenland. A recent study by Choiniere (1992) compared causes of death in 8,000 Baffin Inuit natives with non-native Canadians as a whole. He tabulated seven causes of which we present three here: neoplasms Inuit 23%, Canadians 26%; respiratory system disease Inuit 19%, Canadians 8%; circulatory system disease Inuit 11%, Canadians 43%. Once again it appears that a diet of n-3 FAs protects these natives from cardio-disease in spite of the fact that their habits include heavy smoking. In his study Bang *et al.* (1971) showed that the male Inuit in Greenland had significantly higher

levels of HDL cholesterol; this apparently reduces the risk of cardiovascular disease (Pederson *et al.*, 1996). Presumably, their diet of n-3 FAs or possibly some genetic factor as well protects cold-weather natives (Dyerberg *et al.*,1975).

Mechanism of action of n-3 and n-6 FAs: To understand the probable protective action of n-3 FAs, it is necessary to consider the "cascade" as the original materials eaten by the mammal (shorter chain unsaturated acids) are converted to longer chain n-3 FAs. From plant material the 18:3 n-3 linolenic acid is eaten by mammals (Sanders, 2000). Elongation and desaturation steps convert this 18 carbon FA to 20:5 n-3 a compound called EPA. Over two stages this is converted to DHA (22:6 n-3). These long chain unsaturated FAs are competitive inhibitors of cyclo-oxygenase and lipoxygenases and down regulate eicosanoid synthesis from arachidemic acid. Significantly, these long chain n-3 FAs regulate platelet function (Thorwest *et al*, 2000). In this case, their action is inhibitory and may explain the protective action of these materials in combating cardiovascular disease. The clumping of platelets is related to the formation of coronary clots, and it is probable that this formation is inhibited by the long chain polar bear n-3 FAs in question (Weber and Raederstorff, 2000). Although these materials may protect polar bears against cardiovascular disease, they are not protected against gallstones. Polar bears have in their bile ursodeoxycholic acid. This makes cholesterol more soluble, and it is used in the clinic to treat gallstones. However, its presence in bears does not entirely prevent gallstones. The junior author did a necropsy on a 300kg 10-year-old polar bear at Point Barrow and found the gall bladder almost entirely filled with one large gallstone.

Two controversies in this field: One school of thought favors two groups of FAs as providing protection against cardiovascular disease (Connor, 2000; Kris-Etherton *et al*, 2000). Mulvad (1996) and coworkers suggest that the Greenlanders who they studied having low ischaemic heart disease, are protected by high amounts of long chain monounsaturated FAs. They found high amounts of these in the fatty tissue of Greenlanders when autopsied. Support for their concept is found in our polyunsaturated FAs in both the triglycerides and cholesterol. These FAs were highest in the fed polar bears and may be protective. However, even more striking was the ratio between the fed and fasted bears in the n-3 FAs.

Another controversial matter in this field is whether to use fish-oil capsules high in n-3 FAs in the clinic (Asset *et al.*, 2000; Goodfellow *et al.*, 2000; Nestel, 2000; Smit *et al.*, 2000). Our results showing lowered cholesterol when polar bears have a high n-3 FA level in their blood compared to fasted animals eating no n-3 FAs, give some slight support to the concept of using fish-oil capsules to lower cholesterol.

Footnote*: Abbreviations used: VLDL – very low density lipoproteins; LDL – low density lipoproteins; HDL – high density lipoproteins. Later in the text the fatty acids are abbreviated as number of carbon atoms: number of double bonds. Thus, 20:5 signifies a fatty acid containing 20 carbon atoms and 5 unsaturated bonds. 18: 2n-6 is an omega-6 fatty acid (linoleic) with the first double bond six carbon atoms in from the terminal methyl group.

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Nutritional Value and Microbiological Safety of Fresh Fruit Juices sold through Retail Outlets in Qatar

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Abstract: The nutritional value of ten fresh fruit juices purchased from retail outlets in Doha, Qatar was calculated on the basis that approximately 100 g of fruit is used to make one glass of juice (250 ml). Avocado juice was the best source of energy and potassium followed by banana juice, while guava juice was an outstanding source of vitamin C and carotene. By contrast, the microbiological quality of all the products was well outside the Gulf Standards for fruit juices, and coliform counts usually exceeded 1,000 cfu ml^{-1}. In one sample of mixed fruit juice, the coliform count was above 1.0 x 10^6 cfu ml^{-1}, and both *Escherichia coli* and *Enterococcus faecalis* (1.0 x 10^7 cfu ml^{-1}) were detected. It is concluded that, while the practice of consuming fresh fruit juices with meals should be encouraged on nutritional grounds, steps must be taken to improve the microbial quality of the products.

Key words: Fruit juices, nutritional value, microbiological quality

Introduction

In Qatar, as well as in most countries in the Middle East, the hot climate means that the intake of liquids must be high to compensate for the inevitable losses from perspiration and respiration. Even in air-conditioned buildings, the need for cool drinks is unavoidable. At one time, cool drinks purchased alone or with meals tended to be in factory-filled cans or bottles but, recently, two additional sources of 'water' have become important. Locally-bottled or imported spring water has become widely popular but, for more social occasions, fresh fruit juices are often the beverages of first choice. Most restaurants, cafes and, even, road-side stalls have on-site facilities for extracting the juice from fresh fruits like oranges, mangoes and any other fruits that may be available, and then serving the juice, liberally dosed with ice, to their thirsty customers.

Such drinks have much to recommend them. They are extremely pleasant on the palate and they contain most of the minerals and vitamins found in the original fruit but, bearing in mind their method of extraction, an inevitable question arises over safety. For example, the outside of the fruit may not be washed before it is placed in the juice extractor and, even if it is washed, the total colony count may well exceed 1.0 x 10^5 colony-forming units (cfu) cm^{-2} (Splittstoesser, 1979; Harrigan, 1998). Clearly many of these micro-organisms will be harmless yeasts and saprophytic bacteria, but this confidence does not mean that pathogens like *Listeria monocytogenes* or *Escherichia coli* may not be present as well (Bryan, 1977). How many of these micro-organisms will enter the juice itself will vary with the system of extraction, but it is more than likely that some degree of contamination always occurs.

Then the glasses and the fruits have to be handled by the operator of the extraction system so that, if high standards of hygiene are not observed, faecal coliforms could contaminate the drink along, perhaps, with *Staphylococcus aureus*. The use of tap water from a poorly-maintained storage tank or water from a bore-hole to fill the glass to the required volume may pose an additional risk. Thus, both *Aeromonas hydrophila* (Fernandez *et al.*, 2000) and *Cryptosporidium parvum* (Levine *et al.*, 1991; Mazounie *et al.*, 2000) have been detected in Municipal Water Supplies, while *L. monocytogenes* and *Yersinia enterocolitica* have been isolated from drinking water from wells (Korhonen *et al.*, 1996). Equally relevant could be the finding of Wang and Doyle (1998) that *E. coli* 0157 could survive in a viable state in water for 12 weeks at 25°C and, although in a non-culturable condition, there was no evidence to suggest that the same cells could not have been infective.

A further aspect of concern is the quality of the fruits themselves for, as the juice is the only component examined closely by the consumer, it would be comparatively easy to make use of sub-standard fruit. Infections with moulds like *Penicillium expansum* could easily pass unnoticed, and yet entry of the mycotoxin, patulin, into the juice would represent a long-term risk that should not be ignored (Sharma and Salunkhe, 1991).

Table 1: Potential nutritional value of samples of fresh fruit juices purchased from retail outlets in Qatar; all figures [g (kJ - energy) in a 250 ml portion] based on the inclusion of 100 g of whole fruit pulp/juice made up to 250 ml of juice as consumed

Type of Juice	Protein	Fat	Carbohydrate	Energy	Fibre*
Apple	0.4	0.1	11.8	199	1.8
Avocado	1.9	19.5	1.9	784	3.4
Banana	1.2	0.3	23.2	403	1.1
Carrot	0.6	0.3	7.9	146	2.4
Guava	0.8	0.5	5.0	112	3.7
Lemon	1.0	0.3	3.2	79	NA
Mango	0.7	0.2	14.1	245	2.6
Orange	1.1	0.1	8.5	158	1.7
Pineapple	0.4	0.2	10.1	176	1.2
Strawberry	0.8	0.1	6.0	113	1.1

*Determined by Englyst Method N.A = Not Available
(After: Holland *et al.*, 1991).

Consequently, the aim of this survey was to: (a) collect samples of freshly extracted juices from restaurants and cafes around Doha, the capital of Qatar; (b) calculate, on the basis of literature values and the approximate weight of fruit used per glass of juice, the likely nutrient content of certain selected juices; and (c) examine the juices for total colony count, coliforms, *E. coli* and yeasts and moulds as indicators of general standards of hygiene, *Pseudomonas aeruginosa* which can be present if the tap water has not been correctly handled at the treatment plant and *Staph. aureus* and *Enterococcus faecalis* as possible indicators of poor personal hygiene among the operatives; *Ent. faecalis* is regarded by some authorities as a better indicator of faecal contamination than faecal coliforms (Harrigan, 1998).

Materials and Methods

Six local restaurants were selected on the basis of size and general appearance with respect to cleanliness, and each restaurant was visited in turn. At each restaurant, two glasses of juice covering the entire range usually available, namely apple, avocado, banana, carrot, guava, lemon, mango, mixed fruit, orange, pineapple and strawberry, were ordered without ice and, on receipt, the juice was decanted into a sterile Duran bottle (500 ml). At the same time, around ten ice cubes were transferred with sterile tongs from the ice-bucket to a wide-mouth sterile screw-cap jar. Both the bottle of juice and the jar of ice were then transported immediately to the Central Food Laboratory in Doha in a cool box held at

Table 2: Potential nutritional value of samples of fresh fruit juices purchased from retail outlets in Qatar; all figures (mg in a 250 ml portion) based on the inclusion of 100 g of whole fruit pulp/juice made up to 250 ml of juice as consumed

Type of Juice	Vitamins			Minerals		
	C	B-group	Carotene	Potassium	Calcium	Iron
Apple	6	0.31	0.018	120	4	0.1
Avocado	6	1.74	0.016	450	11	0.4
Banana	11	1.09	0.021	400	6	0.3
Carrot	6	0.55	8.100	170	25	0.3
Guava	230	0.32	435.000	230	13	0.4
Lemon	58	0.5	0.018	150	85	0.5
Mango	37	0.72	1.800	180	12	0.7
Orange	54	0.65	0.028	150	47	0.1
Pineapple	12	0.5	0.018	160	18	0.2
Strawberry	77	0.72	0.008	160	16	0.4

(After: Holland *et al.*, 1991)

Table 3: Microbiological quality of samples of fresh fruit juices purchased from retail outlets in Qatar; all counts as colony-forming units ml^{-1} of juice - the range of counts found is shown by the figures for 'lowest' and 'highest' in brackets

Type of Juice	Total Colony Count	Coliforms	Yeasts and Moulds
Apple	6.6×10^4	1.4×10^3	1.0×10^4
	(2.0×10^4 - 1.5×10^5)	(20 - 4.0×10^3)	(100 - 2.5×10^4)
Avocado	4.9×10^6	9.3×10^3	4.0×10^4
	(1.8×10^6 - 1.0×10^7)	(8.0×10^3 - 1.0×10^4)	(200 - 2.0×10^5)
Banana	2.2×10^6	3.2×10^3	3.4×10^4
	(1.0×10^3 - 1.5×10^7)	(ND - 3.5×10^4)	(ND - 6.0×10^5)
Carrot	1.2×10^7	9.7×10^4	3.6×10^4
	(1.0×10^5 - 1.5×10^7)	(ND - 2.9×10^5)	(ND - 3.0×10^5)
Guava	1.3×10^5	1.0×10^3	< 10
	(6.0×10^4 - 2.0×10^5)	(110 - 2.0×10^3)	(ND - 200)
Lemon	3.0×10^8	ND	1.0×10^4
	(7.5×10^5 - 6.0×10^8)	(ND)	(ND - 2.0×10^4)
Mango	1.3×10^5	820	3.2×10^4
	(7.0×10^3 - 1.9×10^5)	(ND - 4.0×10^3)	(ND - 5.0×10^5)
Mixed Fruit	5.7×10^6	1.6×10^5	1.0×10^7
	(1.5×10^5 - 1.4×10^7)	(120 - 1.2×10^6)	(600 - 1.0×10^8)
Pineapple	1.5×10^5	1.9×10^3	3.2×10^4
	(9.0×10^3 - 3.0×10^5)	(400 - 4.0×10^3)	(200 - 1.3×10^5)
Strawberry	3.0×10^5	2.7×10^3	3.0×10^4
	(6.0×10^4 - 5.5×10^5)	(ND - 1.6×10^4)	(ND - 3.2×10^5)
Ice*	2.4×10^3	200	ND

* = typical result ND = None Detected

Table 4: The recommended Microbiological Standards for any fruit juice sold in the Gulf Region; all figures per ml of juice as consumed

	Total Colony Count	Coliforms	Yeasts and Moulds
Maximum Count Anticipated	5.0×10^3	10	100
Maximum Count Permitted	1.0×10^4	100	1.0×10^3

Where:

* The number of samples (n) to be examined equals 5.
* None of the 5 samples should have counts in excess of Maximum Permitted Limit; any sample with a count above Maximum Count Permitted shall be designated as "defective".
* No more than 2 out of the 5 samples should have counts in excess of Maximum Count Anticipated; any sample with a count above Maximum Count Anticipated shall be designated as "marginally acceptable".

After: Gulf Standards (2000)

approximately 4°C with sealed polythene bottles of frozen water. At the Laboratory, the bottles of juices and the jar of ice were stored in a refrigerator (4 - 5°C) for around two hours for the ice to melt, at which time both the juices and the ice were examined for total colony count, coliforms and *E. coli*, *Staph. aureus*, *P. aeruginosa*, *Ent. faecalis* and yeasts and moulds. In every case, the counts were determined using the procedures specified in AOAC (1995).

This same approach was adopted on each visit.

Results and Discussion

An estimate of the gross chemical composition of the juices is shown in Table 1, and there appear to be some marked differences between the juices. The high fat content of the avocado juice is not unexpected and, although this is reflected in the high energy value, the energy to be gained from consuming banana juice will be more available; around 85% of the carbohydrate in the banana is in the form of readily metabolisable sugars.

Citrus juices are a good option for gaining vitamin C, for a glass of either orange or lemon juice (Table 2) would provide a level in excess of the current Recommended Daily Allowance (UK-RDA) of 40 mg (Anon., 1994). Strawberry juice, while not being so readily available in restaurants as the citrus juices, can be especially rich in vitamim C as is guava juice, and even consumption of the more popular mango will provide close to the UK-RDA. Vitamins of the B-group are present in most juices as is carotene but, as shown in Table 2, the carrot and guava juices would seem to be the only major sources of carotene.

As far as minerals are concerned, the citrus juices are a useful source of dietary calcium, but the potassium - sodium balance in

the body may well be enhanced by consuming avocado or banana juice. This point could be extremely relevant for the Middle East where the intake of sodium from salty foods like Feta cheese can be quite high.

Obviously it is not suggested that all fruit juices can make a major input to a diet, but they could certainly be recommended ahead of drinks like Coca-cola or Pepsi-cola. However, while the latter drinks are unlikely to pose any microbiological risk for the consumer, fresh fruit juices are, by their very nature, exposed to the risk of contamination during preparation and are not subject to any preservative treatment prior to being drunk. It was for this reason that the same selection was examined for general microbial quality - total colony, coliform and yeast and mould count, as well as for specific pathogens.

The results are shown in Table 3, and means are given for each juice along with range of counts found. The recommended specifications for fruit juices sold in the Gulf Region are shown in Table 4, and it is clear that the total colony counts and coliform counts - with the exception of lemon juice - exceed the expected standards by a considerable margin. However, while these high counts are well in excess of any reasonable specification, e.g. over one million coliforms per ml of Mixed Fruit juice, it is important that they may not necessarily pose a hazard to the health of the consumer.

Thus, a number of genera from within the coliform group are widely found on vegetable tissues and pose no hazard to humans, but it is the possible presence of *E. coli* and *Salmonella* spp. under the same 'umbrella' that is a cause for concern. Melon, for example, is likely to have a pH above 6.0 so that, if a sliced fruit was to be contaminated with *Salmonella*, rapid growth could occur prior to the fruit being blended into a juice (Golden *et al.*, 1993). This concern was highlighted with one sample of Mixed Fruit juice which gave a coliform count of 1.6×10^5 cfu ml^{-1} that included *E. coli*, and a count for *Ent. faecalis* of 1.0×10^7 cfu ml^{-1}. The concomitant presence of *Staph. aureus* (87 cfu ml^{-1}) suggests that this sample of fruit/juice had been severely contaminated by human contact, and clearly this level and type of contamination is totally unacceptable.

As fruits usually carry a rich microflora of yeasts and moulds, it is not suprising, perhaps, that the counts in the juices were often very high ($> 1.0 \times 10^5$ cfu ml^{-1}), and usually well above the Gulf Standard. However, it is relevant that juices of this type are consumed immediately after preparation and that few yeasts are pathogenic to humans, so that the actual health risk from this group should be minimal. Nevertheless, the presence of yeasts and moulds in many of the juices suggests that the practices involved with the handling of the fruits and the extraction of the juices leaves a lot to be desired with respect to hygiene.

The high level of coliforms detected in the ice should not have been present, but the absence of *Ps. aeruginosa* confirms that the water employed to make the ice had been properly treated at source.

Conclusion

The selected nutrient values shown in Tables 1 and 2 suggest that the current trend to buy fruit juices to accompany a meal or snack has much to recommend it. Inevitably, each juice provides a different range of those components that are desirable in a diet. Banana juice can provide a welcome source of readily-available energy as well as boost the intake of potassium, while a glass of guava juice meets the UK-RDA for vitamin C many times over, as well as supplying a level of carotene found in few other sources. However, these natural benefits will be lost if the microbial quality of the products is so low that consumers are placed at risk of contracting a food-borne infection. How the Authorities can combat this threat is another matter, for while the Standards published by the GCC cannot be faulted, ensuring compliance may prove more difficult.

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Genotype Influences Body Composition of Developing Chicken Embryo

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Abstract: The effect of genotype on postnatal efficiencies of chickens has been well documented. However, little is known about the effect of genotype on body composition and metabolic physiology of chickens during embryonic development. To test the hypothesis that even with equalised egg weight at setting during incubation, there could be some effect of genotype on body composition, an experiment was conducted with embryonic chicks of broiler and layer genotypes at four stages of development during incubation (viz. 12, 16, 18, 20thd). Wet weight ($P<0.01$) and dry weight ($P<0.05$) of embryos were higher in broilers compared to layers. Irrespective of genotype, the wet and dry weights increased ($P<0.01$) progressively and significantly from day 12 to day 20. Water content was not found to be affected by genotype but goes progressively down till 20th d of incubation. Body nitrogen concentration was higher ($P<0.05$) in the pre-hatch chicks of broiler vs. layer genotype but stage of development did not significantly influence the value of this parameter. Broiler had higher ($P<0.05$) ether extract than layer prehatch chicks. Ether extract increased progressively and significantly ($P<0.01$) during the entire period of pre-hatch development. Body ash content was neither affected by genotype nor stage of development during embryonic period. This appears to be the first report that demonstrates differences in the body composition of broiler and layer genotype during embryonic life life itself.

Key Words: Genotype, body composition, developing chicken embryo

Introduction

Body composition study is of considerable interest to animal and human health researchers. This is because it has got relation to 1) meat quality in animals and 2) obesity and related metabolic disorders in human beings. Nutritional and developmental physiology during embryonic life is important because any changes during this period can have effect on metabolism with consequent impact on body composition, through changes in efficiency of nutrient metabolism and utilisation. The study on developmental biology of embryonic chicken is of special significance not only for poultry industry but also for biomedical research because nutritional requirements at organ level (such as amino acid requirements of brain) of embryonic chicken and human fetus largely coincides (Gelder and van Belanger, 1989). Studies on chickens have been done that indicate effects of breed, sex, strain, nutrition and or combination of one of these on postnatal growth, carcass composition (Edwards and Denman, 1975; Robbins and Ballew, 1984; Jones *et al.*, 1986) and physiological reasons thereof (Porter, 1998). Some experiments (Al-Murrani, 1978) indicated differences in embryonic and postembryonic growth because of differences in egg size. However, with equalised egg weight at setting, changes in growth and body composition of prehatch chicks of broiler and layer genotypes seems to be not investigated. This study was undertaken to find an answer to the question" With equalised egg weight at setting, does the genotype influences the growth and body composition of prehatch chicks of broiler and layer genotype during embryonic development?"

Materials and Methods

Selection and incubation of fertilized eggs: Fertilized embryonated eggs of commercial broiler and layer genotype of uniform shape, size and approximately the same weight (59 ± 1 g) were obtained from the Phoenix Hatcheries, Jabalpur (MP). They were incubated in a model step up mammoth incubator at 100 °F and relative humidity of 86 ± 1%. The prehatch chicks of four developmental stages studied were in 12, 16, 18 and 20th day stage of incubation. The total pre-hatch chicks were divided into four groups of six each upon genotype at every stage of development. They were killed by decapitation. Study on the ponderal changes and chemical analysis of the whole chick was carried out as per AOAC (1980).

Statistical Analysis of the Data: Analysis of variance of the data on body composition was performed as genotype and stage of development as major factor and interaction between them (Snedecor and Cochran, 1968). The means determined to differ significantly were separated using the least square means procedure and the probability level determining significance was $P \leq 0.05$ or $P \leq 0.01$.

Result and Discussion

The embryonic chick, growing in the ovaparine environment is endowed with the parental genetic heritage and nutritional reserves provided by mother hen. Phenotypic expression of genetic differences may be related to differential rates of assimilation of organic and inorganic constituents from extra-embryonic reserves. Several reports (Romanoff, 1960; Asmar *et al.*, 1972; Prabhu, 1977) attest to remarkable shifts in the rate of transfer of biochemical moieties at different stages of embryonic growth and development. The results of the hypothesis tested indicate that even with equalised egg weight at setting, such differential rates of transfer of moieties are not only (developmental) stage oriented as reported before but are also probably the cause of changes in growth and body composition of broiler and layer genotypes during embryonic life itself. In present report, we describe the normal developmental changes in the body composition of embryonic chicks of broiler and layer genotype at 12, 16, 18 and 20th day of incubation period.

Ponderal changes and chemical analysis of whole prehatch chick: The data on ponderal changes, water percentage and chemical composition in the pre-hatch are summarized in Table 1. Wet as well as dry weight of the pre-hatch chicks was significantly ($p<0.01$) altered by the genotype as well as stage of development. These weights were significantly higher in the broiler vs. layer pre-hatch chicks. Irrespective of genotype, the wet and dry weights increased progressively and significantly from day 12 to day 20. Similar changes were described by Rinaldini (1960); Bray and Iton (1962); Hassan and Nordskog

Table 1: Body composition of broiler and layer genotypes of pre-hatch chicks during different stages of embryonic development

Days	Genotype	Wet Weight (g)	Dry Weight (g)	Water content (%)	Total Nitrogen (g/g dry)	Ether Extract (g/g dry)	Ash (g/g dry)
12	Broiler	5.802	0.525	90.057	0.103	0.156	0.059
	Layer	5.202	0.449	91.348	0.100	0.128	0.047
		5.50^{a}	0.49^{a}	91.15^{c}	0.102^{ab}	0.142^{a}	0.053
16	Broiler	13.193	2.580	80.623	0.112	0.234	0.062
	Layer	11.562	2.241	79.963	0.098	0.205	0.056
		12.38^{b}	2.41^{b}	80.29^{b}	0.105^{ab}	0.220^{b}	0.059
18	Broiler	24.195	5.510	77.288	0.123	0.267	0.060
	Layer	21.762	4.300	78.943	0.100	0.254	0.053
		22.98^{c}	4.91^{c}	78.11^{b}	0.112^{b}	0.261^{c}	0.057
20	Broiler	45.500	12.103	73.476	0.096	0.380	0.051
	Layer	43.279	11.097	74.358	0.095	0.335	0.500
		44.39^{d}	11.59^{d}	73.91^{a}	0.095^{a}	0.357^{d}	0.050
$\bar{x} \pm$ SEM		21.132	4.815	80.757	0.103	0.245	0.111
		1.67	0.190	0.206	0.002	0.007	0.012
P Value	D.F						
Genotype (G)	1	**	*	NS	*	<0.05	NS
Stage (St)	3	**	**	**	NS	<0.01	NS
G x St	3	NS	NS	NS	NS	NS	NS

Each mean on wet weight, dry weight and water content is average of six and each mean on total N, ether extract and ash is average of three observations. Pooled means with in a column with different superscript differ significantly (*$P<0.05$, **$P<0.01$), N.S. = Non significant

(1971); Asmar *et al.*(1972). However, Romanoff (1929) observed relatively slow growth between day 9 and day 16, which, according to him, could be ascribable to variation in the magnitude of biochemical changes. Environmental factors like relative humidity and temperature also appeared to influence the pre-hatch growth profile.

It is noteworthy that even with equalized egg weight at setting, the average wet weight of the pre-hatch chicks (data pooled over stages) was significantly higher in the broiler vs. layer genotype. Halbersleben and Mussehl (1922) found that chick weight was related to the weight of eggs, and averaged about 65%. Byerly (1930) observed that the weight or pre-hatch chicks varied, albeit within limits, in the different breeds even with equal sized eggs. Hardin (1972) stated that breed differences in the weight of pre-hatch chicks were not merely associated with differences in the average egg weight, but reflected true genetic differences. Shanawany (1984) showed clear positive co-relation between egg weight at setting and embryo weight at 18th day of incubation and hatchling weight. With the same egg weight, no effect on embryo weight was reported.

The percentage of water decreased significantly at day 16 vs. day 12 but remained virtually unaltered up to day 18. It again registered a significant decrease at day 20. Overall, the percentage of water in whole chick significantly declined during pre-hatch development. This decrease was conspicuous between day 12 and day 16. The apparent decrease at day 18 was, however, not significant. A noteworthy decrease in the percentage of water occurred at day 20. These variations might be related to differential rates of cell maturity. Similar observations were recorded by Romanoff and Romanoff (1967).

A significant ($p<0.05$) effect of genotype on total N concentration was found. However, the stage of development did not significantly influence the value of this parameter. The values of total N concentration (on dry weight basis) in the pre-hatch chicks in the present study are close to the data compiled by Romanoff and Romanoff (1967). The average N concentration was significantly higher in the pre-hatch chicks of broiler vs. layer genotype. This observation points to superior tissue development in the broiler chicks even before hatch. As stated earlier, egg weight at setting remaining equal, the average wet weight of the broiler pre-hatch chicks was significantly higher than that of the layer pre-hatch chicks. Jones *et al.* (1986) demonstrated differences in the fractional rates of protein synthesis between broiler and layer chicks at two weeks old birds.

Ether extract showed a significant effect of genotype ($p<0.05$) as well as the stage of development ($p<0.01$). An effect opposite to the nitrogen, ether extract increased progressively and significantly during the entire period of pre-hatch development. The findings of Speake *et al.* (1993) are noteworthy in this context. They reported major increases in the activity of lipoprotein lipase in adipose tissue and heart from day 12 of development, concomitant with the beginning of the period of lipid uptake from the yolk. The lipoprotein lipase hydrolyses circulating triglycerides to free fatty acids which are either stored in the form of fat in the body or undergo oxidation in the muscle. The significantly higher average value of ether extract recorded in the broiler vs. layer pre-hatch chicks is also noteworthy. This is in consonance with the inherent superior fat synthesizing ability in the former, which is well known fact. Compared to layer strain, growth of abdominal fat pad and its greater lipoprotein lipase activity is a major cause for rapid growth in broiler chicken (Griffin *et al.*, 1987)

During embryonic stages, variation in the normal developmental maturity is dictated by relative proportions of yolk and albumen and is largely attributed to albumen content (Hill, 1993; Finkler *et al.*, 1998; Peebles *et al.*, 2001). Albumen is the primary source of water (determinant of body mass) in the egg and is primary determinant of hatchling size (Finkler *et al.*, 1998). As all the eggs (of similar size) were originating from the flock reared under same managerial and feeding conditions, the differences between composition particularly albumen can not be expected.

The changes in the body composition described above also correspond to the metabolic organs development. Several reports from our laboratory (Pal *et al.*, 1991a; Pal *et al.*, 1991b; Pal and Parmar, 1995; Pal *et al.*, 2002a) on rapid proliferation of liver, thymus, brain and various physiological and morphological changes that occurs during the course of development in these tissues support present findings. More recently, Pal *et al.* (2002b) have reported higher activities of gluconeogenic enzymes in liver and brain of developing chicken embryo which probably reflects higher demand for observed deposition of protein and fat in broilers due to its rapid development, compared to layers embryo. The embryos of poults hatching with higher blood glucose concentration has been reported to grow at faster rate than with low blood glucose level (Christensen *et al.*, 2000b).These authors(Christensen *et al.*, 2000a) also reported that the embryonic growth differs evenwhen not mediated by egg size and functional characteristics and it is probably paternal factor that influences embryonic growth, which in this context is genotype. Total ash content in the pre-hatch chicks did not exhibit any

significant influence of genotype or stage of development. Calcium mobilization from the yolk appears to be at par in the pre-hatch chicks of the two genotypes. Demonstration of hepatic alkaline phosphatase activity of virtually the same magnitude is pertinent in this context (Pal *et al.*, unpublished data).

It can be noted from Table 1 that the major growth and changes in body composition of prehatch chicks occurs during late stages of embryonic development which corresponds to metabolic ontogenesis of equivalent magnitude (Pal *et al.*, 2002b; Pal *et al.*, unpublished data). When compared to other species, these changes resemble to rapid growth and metabolic development of fetus during the last third of generation (Hocquette *et al.*, 2000; Jadhao *et al.*, 2001)

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Chefs' Perception of the Importance of Nutrition in Menu Planning

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Abstract: This study surveyed chefs attending the American Culinary Federation Chefs Forum 2001. They were surveyed regarding their perceptions of the role of nutrition in menu planning. The results showed that chefs strongly agree that food service professionals view nutrition as important in menu planning. The chefs, however, did not perceive that the number of customer requests for modified menu items was increasing or that consumers consider nutrition an important factor when selecting a restaurant. The study found that the chefs' personal health conditions, length of work experience, and recent nutrition education were significantly related to nutrition issues in menu planning. The survey also indicated that chefs no longer perceive that the preparation of low-fat foods requires additional work, and that they can be made equal in taste to foods containing higher amounts of fat.

Key words: Chef, nutrition, menu planning

Introduction

Consumers and foodservice operators view eating out as a necessity with today's fast-paced lifestyle (Spence, 1995; Strauss, 1994). According to a report issued by the United States Department of Agriculture (USDA) (Kantor, 1998), more than two out of three adults say that going out to a restaurant with family or friends not only offers an opportunity to socialize, but optimizes their time by dispensing with cooking and cleaning tasks. The frequency of eating away from home has risen by more than two-thirds over the past two decades and commercially prepared food accounts for 34% of the typical person's total calorie intake (Hunter, 2000). It is anticipated that the upward trend of eating commercially prepared meals will continue in the foreseeable future.

At the same time that the number of meals consumers eat away from home is on the rise, the overall nutritional quality of the typical American diet is on the decline. The United States Department of Agriculture(USDA) (Lin *et al.*, 1999) reports that the nutrient content of meals consumed away from home is failing to keep pace with the nutritional improvements in home-prepared meals. Compared with home-prepared foods, commercially prepared foods have greater amounts of dietary components, such as saturated fat and calories, which Americans over-consume, and less of the nutrients, such as calcium and fiber, that are under-consumed.

This excessive consumption of fat and calories from commercially prepared meals is linked with America's obesity epidemic. In summarizing recent articles published in the Journal of the American Medical Association, Jonas (2001) reports that 63% of men and 55% of women are overweight. In the past twenty years, the prevalence of adult obesity has increased from 14.5 to 22.5%. Type 2 diabetes, gallbladder disease, coronary artery disease, osteoarthritis, hypertension, and elevated serum cholesterol are from 50 to 500% more common in obese individuals than normal weight people. Obesity is related to the consumption of commercially prepared foods and portion size plays a substantial role. A Tufts University Diet and Nutrition Letter survey found that serving sizes at a variety of popular restaurant chains far exceeded USDA guidelines (Linder, 2001).

Nutrition and the Restaurant Industry: Four out of ten deaths in this country are attributed, at least in part, to poor diet and lack of exercise (Dinkins, 2001). Given that the American public consumes 1 billion commercially prepared meals each week (National Restaurant Association, 2000), eating away from home has a tremendous impact on overall health. One goal of The Healthy People 2000 national health promotion and disease prevention program is to "increase to at least 90 percent the proportion of restaurants and other institutional food service operators that offer identifiable low-fat, low-calorie food choices, consistent with the Dietary Guidelines for Americans" (Healthy People, 2000). Some restaurants have responded by offering healthy menu items ranging from low-fat tostados to full-course meals featuring seafood or chicken dishes that are low in sodium and fat but high in fiber and vitamins (Kurtzweil, 2000; Wenzel *et al.*,1999).

The nutrition expertise of chefs is a key component in the continuing effort to convince consumers to change their eating habits and to seek out healthy food items when eating out. To accomplish this, consumers must adapt home eating habits to the commercial environment. Nutrition education for chefs is crucial if restaurants are to stay competitive in the future, as studies have shown that healthful food will be accepted by customers only if the food appeals to the senses, looks exciting and tastes good (Rouslin and Vieria, 1998).

Reichler and Dalton (1998) found that although chefs were practicing some healthful food preparation techniques, the factors of time, taste and training still posed barriers. For example, more than 50% of the chefs surveyed in this study agreed or strongly agreed that recipe modification was time consuming, and only 39% agreed or strongly agreed that food would taste good if current dietary guidelines were followed. The chefs acknowledged having responsibility for the nutrient content of the dishes prepared and providing nutrition information to patrons. The authors suggest that chefs and dietitians work together in food service settings to create foods that not only meet the dietary guidelines, but also enhance customer satisfaction with modified menu items.

Rouslin and Vieira (1998) found that chefs are becoming more nutrition aware and responsive to customers' demands for healthful menu items. A survey conducted by Fitzpatrick *et al.* (1997) found that customer satisfaction with lower-fat items was significantly greater than satisfaction with their higher fat counterparts, regardless of the menu-item type, dining experience, or respondent characteristics. But the majority of restaurateurs still report that although customers say they want healthier menu items, they do not consistently select healthful menu items (Jones,1999).

Studies concerning the importance of nutrition in the consumers' selection of commercially prepared foods show conflicting results. The chefs surveyed by Reichler and Dalton (1998) did not think that customers were concerned about nutrition. A National Restaurant Association study reported in Frozen Food Digest (Wenzel, 1994) found that 55% of respondents considered the

nutritional content of food when eating out whereas a similar survey conducted by the Center for Science in the Public Interest found that 74% of adults considered healthy choices an important factor when selecting a restaurant (Lewis, 1994).

Restaurants are now and will continue to be a major source of food and nutrition for the American public. This escalating trend highlights the importance of the chef's role in offering and preparing healthful food. Consequently, this study was designed to examine current attitudes of chefs regarding the importance of nutrition in menu planning in today's food service establishments.

Materials and Methods

A questionnaire was developed by revising and expanding the survey of Palmer and Leontos (1995) that examined chefs' perception of nutrition before and after completing a series of nutrition education classes. The 2-part questionnaire administered in this study was contained 26 questions. The first part of the questionnaire addressed demographic information, including age and gender, and asked questions about the type of establishment in which chefs currently worked, the region of the country they came from, and whether they had received nutrition education during the last 5 years. The second part of the questionnaire included 16 questions measuring the chefs' attitudes toward low-fat menu items, healthy food preparation practices, the role of nutrition in the planning of menus, and the importance of nutrition for the maintenance of an individual's health. The survey was distributed to chefs participating in the American Culinary Federation Chefs Forum 2001, in Las Vegas, Nevada. Two hundred and fifty questionnaires were distributed and 181 were returned. One hundred and seventy-nine responses were deemed valid for data analysis for a total response rate of 72%. Table 1 shows the characteristics of the sample.

Table 1: Respondents' Profile

Item	Frequency	Percent
Age		
(31-34)	40	22.1
(41-50)	63	34.8
(51-60)	63	34.8
> 60	13	7.2
Total	179	98.9
Missing	2	1.1
Gender		
Male	140	77.3
Female	36	19.9
Total	176	97.2
Missing	5	2.8
Number of years of experience		
5-10	23	12.7
11-15	25	13.8
16-20	44	24.3
> 20	86	47.5
Total	79	89.9
Missing	2	1.1
Type of establishment		
Quick Service	1	0.6
Buffet	1	0.6
Family	6	3.3
Ethnic	3	1.7
Gourmet	14	7.7
Specialty	4	2.2
Education	96	53.0
Healthcare	9	5.0
Industrial	6	3.3
Other	39	21.5

Seventy percent of the chefs were in the range of 41-60 years of age and 48% had more than twenty years of experience in the food service industry. The sample was distributed geographically throughout the country. Approximately half of the chefs were currently employed in a college or university setting.

The Statistical Package for the Social Sciences (SPSS) program was used to calculate the mean responses for chefs' attitudes about the role of nutrition in their work. A series of analysis of variance (ANOVA) was performed to identify the existence of significant differences among different backgrounds and demographic factors in relation to nutrition attitudes and practices. If any significant differences were found, a *post hoc* multiple comparison procedure using the Tukey method was used to further investigate significant differences among different groups based on respondents' background variables (i.e., gender, age, educational level, work environment, nutrition education, personal health history and type of restaurant establishment).

Results

Table 2 shows the descriptive analysis regarding chefs' perceptions about the role and importance of nutrition in their personal lives and in the restaurant environment.

Respondents strongly agreed with only items: "Nutrition is taken into consideration when planning menu items (4.5 on a 5-point scale)" and "Consumers take nutrition into consideration when making menu choices (4.3)."

Respondents displayed a high agreement toward the following items: "I try to cut down the fat in my own diet (4.0);" "I like the challenge of making low-fat food taste good (4.0);" "Consumers are concerned about fat in their diet (3.9);" and "People want low-fat choices on restaurant menus (4.0)," and "Nutrition plays an important role in the development of chronic disease (4.0)."

The responses to "The frequency of restaurant eating impacts an individual's nutritional health (3.7)," and "Customers with special nutritional needs can select appropriate items from our menu (3.9)" ranked between neutral and agree.

Two items received lower mean scores than the rest of the items: "Cooking low-fat items is more work than it is worth (2.1)," and "Low-fat food does not taste as good as high-fat food (2.7)." These ratings indicate disagreement with the statements; in other words, chefs actually have a positive attitude toward the ease of preparation and taste of low-fat foods. Only two items generated neutral opinions: "Low-fat food does not taste as good as high-fat food. (3.1)"; and "The number of customer requests for modified menu items is increasing (3.1)."

In general, the respondents revealed homogenous attitudes about nutrition and there were very few significant differences among different groups based on respondents' background variables (i.e., gender, age, educational level, work experience, and type of restaurant establishment). The homogeneity of the responses may in part be attributed to the fact that the majority of the chefs in this sample, 53%, worked in an educational, rather than operational, capacity. Another explanation is that through education and continual training chefs in the American Culinary Federation share a common body of nutrition knowledge and have developed similar attitudes toward the importance of nutrition.

There were only three demographic variables that showed a significant relationship with perceptions regarding the importance of nutrition in personal food choices and in commercial menu offerings: personal health conditions, work experience, and nutrition education. Tables 3, 4, and 5 show the impact of personal health conditions, work experience, and nutrition education on perceptions about the role of nutrition in their personal and professional lives.

Whether or not a chef had experienced nutritionally related diseases, such as diabetes or heart disease, influenced their behavior with regard to the consumption of low-fat food items. For example, chefs who had such health conditions were more inclined to reduce fat in their diet. Further, the level of work experience influenced respondents' opinions about the amount of work required to prepare low fat items. Chefs with work experience of less than 5 years were inclined to agree that low-fat items represent more of an effort to prepare than chefs that had

Table 2: Chefs' perceptions about the role and importance of nutrition in their personal and professional lives

Label	Item*	Mean	S.D.	N
Q 1	Low-fat food does not taste as good as high-fat food.	2.726	1.160	179
Q 2	I would feel comfortable serving a low-fat item even when it was not specifically requested.	3.848	0.9475	178
Q 3	I try to cut down on fat in my own diet.	4.005	0.9087	179
Q 4	Cooking low-fat items is more work than it is worth.	2.061	0.8646	179
Q 5	People like to eat low-fat food.	3.123	1.709	179
Q 6	I like the challenge of making low-fat food taste good.	3.955	0.8198	179
Q 7	Low-fat food does not taste as good as high-fat food.	3.150	0.8511	179
Q 8	Consumers are concerned about fat in their diet.	3.860	0.8853	179
Q 9	People want low-fat choices on restaurant menus.	3.972	0.8574	179
Q 10	Nutrition plays an important role in the development of chronic disease.	3.972	0.8574	179
Q 11	Nutrition is taken into consideration when planning menu items.	4.497	0.7140	179
Q 12	The frequency of restaurant eating impacts an individual's nutritional health.	3.748	0.9352	178
Q 13	Consumers take nutrition into consideration when making menu choices.	4.309	3.923	177
Q 14	The number of customer requests for modified menu items is increasing.	3.141	1.004	179
Q 15	Customers with special nutritional needs can select appropriate items from our menu.	3.921	0.8173	177
Q 16	Customers take nutrition into consideration when selecting a restaurant.	3.666	0.9273	178

*The respondent was asked to use a 5-point Likert-type scale (Strongly disagree = 1; Disagree = 2; Neutral = 3; Agree = 4; Strongly Agree = 5) for all questions.

Table 3: Significant differences regarding health conditions

Item*	Mean		F-value	P-value
	Have heart disease or diabetes (N=35)	Do not have heart disease or diabetes (N=141)		
Q 3	4.34	3.95	5.58	0.019**

* Refer surveyed items to those in Table 2 ** Significant at the 0.05 level

Table 4: Significant work experience differences

Item*	Mean				F-value	P-value
	G 1 (N=23)	G 2 (N=25)	G 3 (N=43)	G 4(N= 85)		
Q 4	2.47	1.84	1.93	2.07	2.82	0.041**

*Refer surveyed items to those in Table 2. Grouping as follows: G1: less than 5 years of work experience, G2: 5-10 years G3: 11-15 years, G4: 16-20 years ** Significant at the 0.05 level

Table 5: Significant differences in regard to nutrition education received

Item***	Mean		F-value	P-value
	Have attended classes within past 5 years (N=119)	Have not attended classes within past 5 years (N=60)		
Q 11	3.84	3.56	3.46	0.064*

***Refer surveyed items to those in Table 1. *Significant at the 0.10 level

more experience. However, in general respondents disagreed with this statement. Finally, the consideration given to nutrition when planning a menu was influenced by the amount of nutrition education chefs experienced recently. Chefs who had attended nutrition classes within the past 5 years were more inclined to consider nutrition in their menu planning process, which is not surprising, as nutrition education is expected to increase their level of awareness.

Discussion

The neutral responses to the statements "The number of customer requests for modified menu items is increasing" and "Customers take nutrition into consideration when selecting a restaurant" have important implications for food service operators, chefs, and consumers. The majority of restaurateurs report that although customers say that they want healthier menu items, their purchasing behavior does not match their stated intentions (Jones, 1999). Taste remains the most important issue when ordering food. Although an increasing number of people display an *interest* in health and nutrition, restaurant patrons still do not consistently translate this interest into selecting healthy menu options or asking for them when they are not presented on the menu. The question remains as to whether this inconsistent consumer behavior represents a lack of personal dietary commitment, or perhaps a lingering negative attitude towards modified menu items. Commercial food service operators may wish to offer more nutrition conscious items, but are unable to do so unless it is profitable. In other words, customers who say they want healthy food must request and order healthy food on a regular basis. Only then will operators find it necessary to modify their menu selections.

This survey showed a significant ($p < .05$) relationship between chefs who have heart disease and/or diabetes and with the reduction of fat intake in their personal diets. The effect of food choices certainly becomes stronger when it impacts one's own mortality. It is not unexpected, therefore, for chefs to make dietary modifications to improve their health. It is also likely that these chefs received dietary directives from medical professionals. Chefs with chronic conditions would serve as powerful examples within the profession of how appropriate nutrition choices and food preparation can prevent and manage life-style illnesses.

The number of years of work experience showed a significant ($p < .05$) difference in attitudes towards the amount of work involved in preparing acceptable low-fat menu items. Chefs with less than five years of work experience were more likely to feel that tasty low-fat items are more difficult to prepare than their higher-fat menu counterparts. This may be attributed to fewer years of general work experience, a lack of opportunity to learn about low-fat food preparation that also has exceptional taste, the inability to work in establishments that encourage low-fat recipe development, or personal inexperience in preparing creative low-fat meals.

In general, however, the chefs surveyed in this study disagreed with the statements that low-fat foods are difficult to prepare and do not taste as good as higher-fat menu items. These results differ from those of Reichler and Dalton (1998), thus possibly

indicating a change in attitude towards low-fat recipe development. Rouslin and Vieria (1998) found that chefs were interested in applying more nutrition principles in commercial food service operations. Palmer and Leontos (1995) found a positive attitude change towards preparing low-fat food items after chefs completed a nutrition education course. These authors, as well as Sims- Bell (1998), conclude that opportunities exist for dietitians to market education that will not only teach nutritional requirements, but also empower chefs to accept responsibility for healthful menu planning.

A significant ($p < .10$) relationship was found between the response to the statement "Nutrition is taken into consideration when planning menu items" and having attended nutrition education classes within the past five years. Nutrition science and its application to practical life are continually evolving. Persons learning about recent advances in nutrition and its relationship to the risk reduction of certain chronic diseases are more aware of the importance of promoting healthful menu choices. This finding emphasizes the importance of providing nutrition education to chefs either via professional association seminars or on-the-job training.

The public's interest in nutrition, combined with the rise in the number of meals eaten away from home and the increase in the incidence of chronic diseases, represents a challenge and responsibility for today's chefs. This study shows that chefs strongly believe that food service professionals and consumers view nutrition as important in menu planning. Experienced chefs no longer perceive that the preparation of low-fat foods requires additional work, and that low fat-foods can taste great. This study found that personal health conditions, amount of work experience, and recent nutrition education were significantly related to nutrition in menu planning issues. These findings support the need for dietetics and culinary professionals to continue to work together to find innovative ways to improve chefs' knowledge and attitude towards nutrition, and to convince the public that making healthful selections when eating out is not only possible, but tasteful and enjoyable. Future research should continue to focus on identifying barriers to healthful eating in the commercial food service industry.

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Tocotrienol - Rich Fraction and its Effects on Parameters Affecting Gastric Mucosal Integrity after a Single Exposure to Indomethacin

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Abstract: The effect of treatment with a tocotrienol-rich fraction (TTRF) on acute single exposure to indomethacin was investigated. Forty-eight male rats of the *Sprague-Dawley* (200-250g) species were randomly assigned into two groups (N and T). The N group was fed with a commercially prepared normal rat diet and the T group was fed with an identical diet enriched with TTRF 150mg/kg diet. Each group was further subdivided into two subgroups that was either challenged (NI and TI) or not challenged with indomethacin (NX and TX). After eight weeks of treatment the NX and TX rats were killed and the stomachs isolated whereas the NI and TI rats were challenged with a single dose of indomethacin (80mg/kg body weight) orally and after six hours the rats were killed. Measurements for malondialdehyde (MDA), glutathione content, PGE_2, gastric acid concentration and gastric adherent mucous (GAM) were done. Gastric PGE_2 content and acid concentration were comparable in the NI and TI groups compared to its corresponding group that was not challenged. The gastric MDA content and GAM concentration were increased in the NI and TI compared to its corresponding group that was not challenged. This indicated that indomethacin increased MDA and treatment with TTRF could not inhibit the rise of MDA whereas TTRF has no effect on GAM concentration. The glutathione ratio was however, only elevated in the TI group compared to the TX, which indicates that in acute mucosal injury by indomethacin, TTRF is able to preserve the ratio of the endogenous antioxidant. We conclude that TTRF has beneficial effects on gastric parameters.

Keywords: TTRF, indomethacin, MDA, glutathione, PGE_2, gastric acid, gastric adherent mucous

Introduction

The non-steroidal anti-inflammatory drugs (NSAIDs) have been among the most widely used drugs and although the non selective NSAIDs have been notorious in causing gastrointestinal lesions, the mechanism of how these lesions are generated have remained obscure. The emergence of the selective NSAIDs is indeed a breakthrough as it ameliorates the gastrointestinal side effects. The use of the non-selective NSAIDs is still popular due to its relatively cheap cost. Hence, studies exploring the possibilities of preserving gastric mucosal integrity during treatment with non-selective NSAID are still being conducted (Sontag *et al.*, 1994; Taha *et al.*, 1996; Hawkey *et al.*, 1998).

The therapeutic effects and major toxic side effects of NSAIDs have been attributed to the ability of these drugs to inhibit the synthesis of prostaglandin (PG), through a direct action on prostaglandin H synthetase, which serves both as a cyclooxygenase (COX) and as a peroxidase (Davies and Wallace, 1997). PGs increase both the synthesis and the release of gastric mucous while NSAIDs has the opposite effects. Free radicals production by NSAIDs is among the more probable mechanism suggested that disrupt the gastric mucosal integrity (Ali *et al.*, 1996; Granger *et al.*, 1986). The body has endogenous antioxidant, which under normal conditions is adequate to protect the organs. In situations that differ from normal such as exposure to noxious stimuli, vulnerable organs such as the lung, liver and stomach need a high level of nonprotein sulfhydryls (mainly reduced glutathione) to maintain integrity (Kosower and Kosower, 1978; Boyd *et al.*, 1979). In such situations, exogenous antioxidant may prove to be beneficial.

Alpha tocopherol (vitamin E) is a naturally occurring antioxidant in the biological systems and is present in the cell membrane of various tissues including intestines and stomach (Granger *et al.*, 1986). The biological activity of vitamin E is generally believed to be due to its antioxidant action rendering it capable to inhibit lipid peroxidation in biological membranes by scavenging the chain propagating peroxyl radicals, thus blocking the free radical chain reaction.

Tocotrienol, the vitamin E that may be obtained from palm oil has been shown to be a better antioxidant (Serbinova and Packer, 1994; Afaf and Appleqvist, 1996). In our efforts to search for avenues to minimise disturbances in the gastric environment due to NSAIDs, this study is carried out to determine the effects of TTRF on important gastric parameters after exposure to indomethacin. This study also investigated the effects of a single exposure of indomethacin on the same parameters.

Materials and Methods

In this study, forty-eight male rats of the *Sprague-Dawley* (200-250g) species were randomly assigned into two groups (N and T). The N group was fed with a commercially prepared normal rat diet and the T group was fed with an identical diet enriched with TTRF 150mg/kg diet. The TTRF enriched diet was prepared by dissolving 150mg of palm oil in a sufficient amount of acetone, pouring it over 1 kg of rat pellet and allowing the acetone to evaporate. The normal rat pellets were treated with acetone only. Each group was further subdivided into two subgroups that was either challenged (NI and TI, each $n = 12$) or not challenged with indomethacin (NX and TX, each $n = 12$). After an eight-week study period the NX and TX rats were killed and the stomachs isolated. Whilst the NI and TI rats were first challenged with a single dose of indomethacin (80mg/kg body weight) orally and were killed only after six hours post challenged. Of the twelve rats in each group, measurements for MDA, glutathione content, PGE_2, and gastric acid concentration were done in six rats while the remaining stomachs ($n = 6$) were used for the analysis of gastric adherent mucous.

The lower end oesophagus and pylorus were clamped and the stomach was removed. Gastric tissue MDA content was measured using a modified method described by Ledwozyw *et al.*, 1988. The gastric tissue was homogenise in distilled water, centrifuged and the diluted supernatant was added with trichloroacetic acid. After 15 minutes at room temperature, thiobarbituric acid was added and the samples were incubated in 100°C water bath for 30 minutes. After cooling, n-butanol was added and the absorbency of the upper phase was read.

Gastric glutathione content was measured using a well-established method (Griffith, 1980) . The gastric tissue was homogenise in 4 volume of 5% TCA/0.01N HCL and centrifuged at 17000 X g for 15mins at 2°C. The supernatant was separated for GSH and GSSG assay. The ratio for reduced glutathione to oxidised glutathione

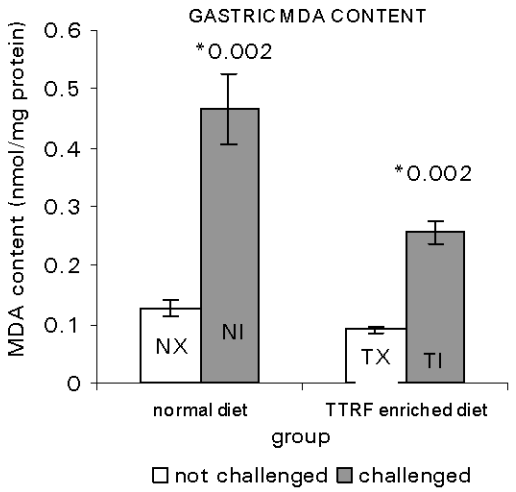

Fig.1: Effects of TTRF and indomethacin on gastric MDA content There was an increase in the gastric MDA content after challenged with indomethacin for both groups (NI and TI) compared to their respective controls (NX and TX) (P = 0.002).

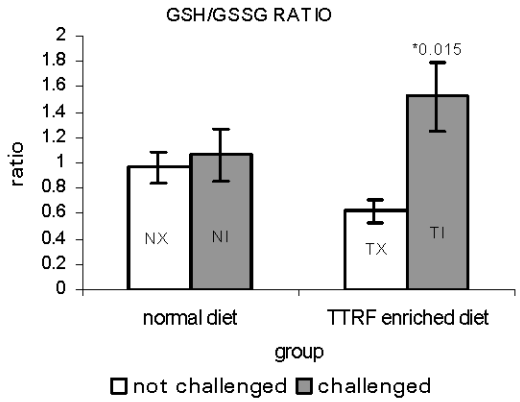

Fig. 2: Effects of TTRF and indomethacin on glutathione ratio. There was an increase in the glutathione ratio in the treated group that was challenged with indomethacin (TI) (P = 0.015) compared to the corresponding control (TX). The glutathione ratio in the normal challenged group (NI) did not differ from the normal unchallenged group (NX).

was calculated.

Samples of gastric mucosal tissue were prepared for prostaglandin analysis according to the method described by Redfern *et al.*, 1987. The extraction of PGE_2 was performed using an Amprep C18 cartridge (Amersham International, UK) and the content was analysed using a kit (PGE_2[125] assay system, code RPA 530; Amersham International).

Samples of gastric juice were collected and centrifuged at 3000 r.p.m. for 10 min. Aliquots of each sample were titrated with 0.01N NaOH to a pH of 7.0. The concentration of hydrogen ion was calculated as described by Shay *et al.*, 1954.

Gastric adherent mucous level was determined by Alcian blue dye binding method as described by Corne *et al.*, 1974. The gaster was isolated and immersed in the Alcian blue solution. After two hours the unbound Alcian blue was removed by washing it twice in 0.25M sucrose solution. The mucous bound dye was eluted by using 0.5M magnesium chloride solution. The samples were added with diethylether and centrifuged. The absorbency of the

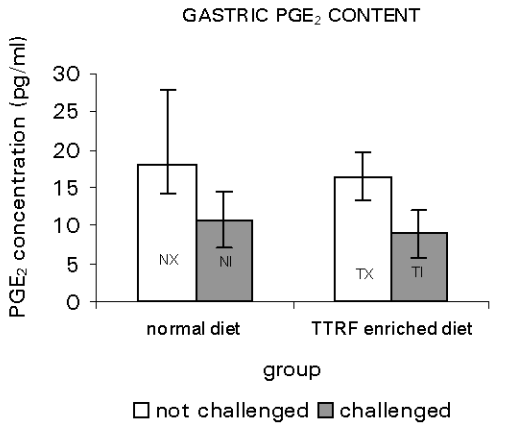

Fig. 3: Effects of TTRF and indomethacin on gastric tissue content of PGE_2. There were no difference in the gastric content of PGE_2 in the normal and TTRF group whether or not the rats were challenged with indomethacin.

aqueous phase was measured using spectrophotometer.

All results were expressed as mean ± SEM. Statistical analysis was performed using t test and a P value of < 0.05 was considered statistically significant for all parameters.

Results

Effect on gastric malondialdehyde (MDA) content: The effects of TTRF and indomethacin on gastric MDA content are shown in Fig. 1. There was an increase in the gastric MDA content after challenged with indomethacin for both groups (NI and TI) compared to their respective controls (P = 0.002). There was a 3.5 fold increment in MDA content for the NI group and 3 fold increment for the TI group compared to the treated, unchallenged group. On a percent basis, the increment in MDA is smaller in the treated group (TI).

Effect on glutathione ratio: The effects of TTRF and indomethacin on glutathione ratio are shown in Fig. 2. There was an increase in the glutathione ratio in the treated group that was challenged with indomethacin (TI) (P = 0.015) by 2.5 fold compared to the corresponding control (TX). The glutathione ratio in the normal challenged group (NI) did not differ from the normal unchallenged group (NX).

Effect on gastric tissue content of PGE_2: The effects of TTRF and indomethacin on gastric tissue content of PGE_2 are shown in Fig. 3. The gastric tissue content of PGE_2 in the NI group did not differ from the unchallenged group (NX). There was also no difference in the gastric tissue content of PGE_2 in the treated group whether or not the rats were challenged with indomethacin. This also demonstrated that indomethacin did not have an effect on gastric tissue content of PGE_2 as the content remain unchanged in the untreated group (NI), compared to whether or not challenged (NI Vs NX).

Effect on gastric acid concentration: The effects of TTRF and indomethacin on gastric acid concentration are shown in Fig. 4. The gastric acid concentration in the NI group did not differ from the unchallenged group (NX). Similar observations were made in the groups treated with TTRF that is there was no difference in the gastric acid concentration in the TTRF group whether or not the rats were challenged with indomethacin. This also demonstrated that indomethacin did not have an effect on gastric acid concentration as the concentration remain unchanged in the untreated group (NI), compared to whether or not challenged (NI Vs NX).

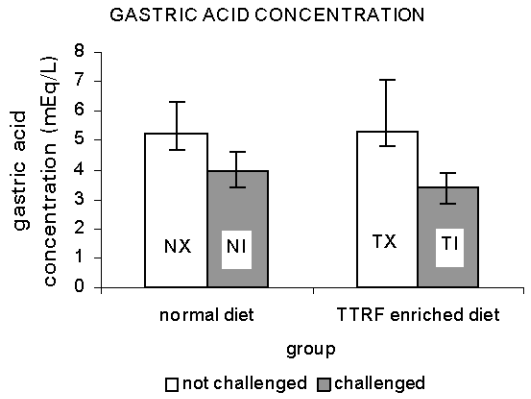

Fig. 4: Effects of TTRF and indomethacin on gastric acid concentration. There were no difference in the gastric acid concentration in the normal and TTRF group whether or not the rats were challenged with indomethacin.

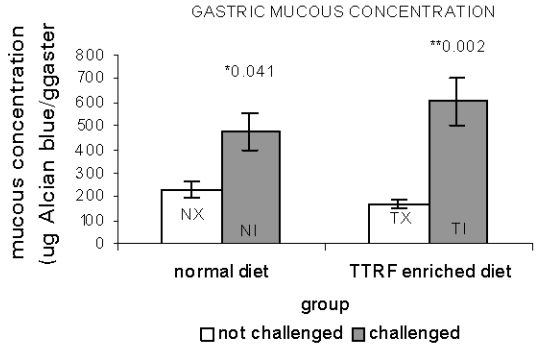

Fig. 5: Effects of TTRF and indomethacin on gastric adherent mucous quantity There was an increase in gastric mucous quantity after challenged with indomethacin for both normal (NI) and the TTRF groups (TI) (P=0.041 and P=0.002 respectively) compared to their corresponding controls

Effect on gastric adherent mucous quantity: The effects of TTRF and indomethacin on gastric adherent mucous quantity are shown in Fig. 5. There was an increase in gastric mucous quantity after challenged with indomethacin for both normal (NI) and the TTRF groups (TI) (P=0.041 and P=0.002 respectively) compared to their corresponding controls. The GAM quantity double in the NI group whilst a 3.5 fold increment was observed in the TI group compared to before challenged with indomethacin.

Discussion

Just as many factors are involved in the maintenance of gastric mucosal integrity, factors causing the injury are also many and divers. Some common identified aggressive factors include smoking, drugs such as NSAIDs and steroids, Helicobacter pylori, acid and pepsin. Although the gastric mucosa has protective factors such as adherent mucous layer, bicarbonate, phospholipids, prostaglandin and antioxidants, there are situations whereby these protection is breached. Given the widespread use of NSAIDs and their adverse effects on the gastric injury, studies such as the current one is indeed required.

Studies have shown that free radicals are involved in the development of mucosal damage by NSAIDs (Pihan *et al.*, 1987; Naito *et al.*, 1995). These excessive free radicals induce lipid peroxidations which is believed to be an important cause of destruction and damage to the gastric cellular membrane. In the current study, we found that MDA was increased after challenged with indomethacin. Elevated gastric MDA reflects an intensification of lipid peroxidation process. Antioxidants such as TTRF used in this study is expected to retard lipid peroxidation process but this was not the case in our study. Even though TTRF is unable to inhibit the rise of MDA, on a percent basis the increment in MDA is smaller in the treated group (TI) compared to the untreated group (NI). Amongst the factors causing the lack of antioxidant effects of TTRF is the dose of indomethacin used. If, in fact the dose of indomethacin used is high and indomethacin increases the production of free radical, it is highly possible that the amount of TTRF used is insufficient to scavenge the excessive free radical. Hence, it is possible to increase the dose of TTRF in future studies.

In contrast to the findings on MDA, interestingly we found that another indicator of antioxidant status that is the glutathione ratio, increased in the TTRF group that was exposed to indomethacin. Similar changes were not seen in the TTRF group that was not challenged to indomethacin. These observations suggest that TTRF on its own does not increased glutathione synthesis or its production. The ratio is enhance, however only after exposure to indomethacin which indicates that TTRF is able to scavenge the free radical and this reduce the consumption of reduced glutathione (GSH).

Studies have shown that chronic exposure to indomethacin suppressed the gastric prostaglandin synthesis (Redfern *et al.*, 1987; Shorrock and Rees, 1992). In this current study, there was no difference in gastric PGE_2 content. It is therefore evident that a single dose of indomethacin does not inhibit prostaglandin synthesis after a single exposure. Complete inhibition of COX leading to reduce in PGE_2 content will consume a much longer duration and will probably not be seen in a single dose only after 6 hours. The PGE_2 measured is most probably the pre-formed PGE_2. Chronic treatment with indomethacin may lead to irreversible inhibition of PGE_2, in which case the reduced in PGE_2 coupled with lipid peroxidation may caused sustained gastric injury.

Acid an aggressive factor that will ultimately leads to gastrointestinal lesions. Current treatment of the GI lesions employs anti secretory agents that is the H_2 receptor antagonist, proton pump inhibitor and antimuscarinic agent. This study showed that there was no significant changes in acid concentration after exposure to a single dose of indomethacin either in the normal or the TTRF treated group. Previous studies done (Feldman and Colturi, 1984; Wagner *et al.*, 1995) showed that long term exposure to indomethacin led to a significant increase in mean gastric acid concentration. Exogenous prostaglandin has been shown to inhibit basal and stimulated acid secretion in man and animals (Levine and Schwartzel, 1984). As mentioned above, our study showed no changes in the gastric PGE_2 content. This might explained why there were also no changes in the acid secretion after exposure to indomethacin.

After indomethacin exposure, there was increased in gastric adherent mucous content whether or not groups were treated with TTRF. A previous study done also showed that TTRF does not stimulate gastric mucous production (Nafeeza *et al.*, 2000). This showed that the increased in mucous content is probably not due to TTRF but may be the response of a protective mechanism in the gastric environment so as to minimise injury caused by indomethacin. Mucous released in response to topical application of an irritant, played an important role in the repair of epithelial damage through the process of restitution.

We found from this study that TTRF has no effect on PGE_2 content, gastric acid and GAM concentration. TTRF was not able to inhibit the rise of MDA content by indomethacin but able to preserve the ratio of the endogenous antioxidant that is the glutathione. We conclude that TTRF has beneficial effects on gastric parameters.

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Sanitary Quality of Commercially Produced Ice Cream Sold in the Retail Stores

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Abstract: The sanitary quality of commercially produced ice cream of Milkvita, Igloo, Pollar and Savoy were examined for total bacterial count (TBC), coliform count and Staphylococcal count. Each brand of ice cream was collected from retail stores of Dhaka City. The TBC was determined on plate count agar incubated at 30°C for 3 days, coliform count on violet red bile agar incubated at 30°c for24 hours and Staphylococcal count on mannitol salt agar plates incubated at 37° C for 24 to 48 hours. The TBC reported in ice cream of Milkvita, Igloo, Pollar and Savoy varied from 2800to 3800 with an average of 3280 CFU/ml, 2800 to 4000 with an average of 3450 CFU/ml, 10,000 to 19,000 with an average of 15,000 CFU/ml and 26,800 to 56,000 with an average of 42,460 CFU/ml respectively. The coliform count varied from 4 to 18 with an average 11.60 CFU/ml and 18 to 42 with an average of 28 CFU/ml in Polar and Savoy but in case of Milkvita and Igloo, it was found negative. The Staphylococcal count in the samples of Igloo and Savoy varied from 2 to 7 with an average of 3.80 CFU/ml and 7 to 17 with an average of 11.20 CFU/ml respectively, whereas it was found negative in the samples of Milkvita and Polar.

Key words: Coliform count, Staphylococcal count

Introduction

Ice cream is a milk product, which contain a variety of ingredients in addition to milk, cream and sugar. This is a popular dairy product throughout the world. As a result, its production and consumption are rapidly increasing and the substantial part of milk produced in many countries is being utilized for the manufacturer of frozen dessert. The richness in nutritive constituents of ice cream although has been realized by all but the problem lies in the production and handling of this food is vary complex one and is associated with so many problems. So, there are great difficulties in regards to quality and quality of ice cream and microbiological quality of ice cream is also far from satisfactory. At many points, during production, transportation, storage and preparation milk food for consumption, it may become contaminated with biological agents. The biological agents contaminated with in food are traced to ingredients added post pasteurization and environmental factors such as air, faults in storage tank, cracks in the plant and packaging materials (Bigalke and Chappel, 1984). In developed countries ice cream receives quality control measures to increase its shelf life as well as prevent potential threat of public health. Bangladesh is still backward in this respect. Due to non-enforcement of inspection act and lack of maintenance of standard relation to hygienic quality of ice cream, the consumers of this country are deprived of getting quality ice cream. In our country, some commercial company has been marketed ice cream in the local market. The microbiological status of ice cream for public health significance in Germany is known (Furh,1986) but such type of investigation is not known in Bangladesh. The present work was conducted to:-

a) find out presence of selected microbial groups in samples obtained from different brands of ice cream
b) determine and ascertain the sanitary quality of commercially produced ice cream in Bangladesh and compared it with international standards.
c) identify the possible cause of quality deterioration influencing public health hazard.

Materials and Methods

Four different brands of commercially produced ice cream available at retail stores were selected for the study. These brands were Milkvita (Bangladesh Milk Product Co-operative Union Ltd., Dhaka), Igloo (Abdul Monem Ltd., house of Igloo, Dhaka, polar (Dhaka Ice cream industries Ltd., Dhaka) and Savoy (Savoy Ice cream Factory, Sks Product, Dhaka). Three cups of ice cream of each brand were collected from the local market of Dhaka City using sterile containers, which were kept in iceboxes and brought to the laboratory within 30 minutes of collection. A quantity of about 10ml of liquid ice cream was pipetted out from different depths and transferred into a sterile glass bottle fitted with a screw-capped stopper. Three cups of ice cream of each brand were handled as above and the samples were taken in the labeled bottles. In this way, a total of 30 ml of the ice cream sample was collected from each brand. The collected ice cream was considered as a single representative sample. From this thoroughly mixed sample, an exact quantity of 1 ml of ice cream was pipetted out aseptically and transferred into a sterile empty test tube and plugged with cotton. To this ice cream 9 ml of dilution was added to give a 1:10 dilution v/v. Further, decimal dilution as required were prepared according to standard method given by APHA (1960). The prepared samples were bacteriologically examined in order to determine the total viable count of bacteria present in ice cream as well as to detect and enumerate Coliform and Staphylococcui. The media employed for total bacteria counts, Coliform and Staphyloccocci counts were plate count Agar (PCA), Violet Red Bile Agar (VRB) and Mannitol salt agar (MSA) respectively, as described by APHA (1953,1958,1960). The test sample poured on PCA plates were incubated at 30°C for 3 days, VRB plates were incubated at 30°c for 24 hours and MSA plates were incubated at 37°C for 24 –48 hours.

Results and Discussion

The bacteriological status of four brands of ice cream is present in Table-1. The mean TBC in use samples of ice cream of Milkvita (3280CFU/ml) was found lower in comparison to use samples of Igloo (3450 CFU/ml), Polar (15,000 CFU/ml) and Savoy (42,460 CFU/ ml). These findings support the results of Marino (1954) and Keller *et al.* (1987) who were suggested that use fresh ice creams contained not more than 1,00,000 CFU/ml of total bacterial count (TBC) per ml. From the above findings, it was revealed that all brands of ice cream samples were within acceptable limit of public health safety because the samples did not exceed the total viable count 1,00,000 CFU/ml which were in agreement with that of Marino (1954) and Hankin *et al.* (1984). It is clear from the overall results that ice cream samples of milk vita were of the superior most quality, because the counts of total bacteria were less than recommended microbiological standard of Food and Drug Administration and USPHS (1965). Rossi (990) reported that the ice cream might be contaminated due to improperly cleaned servers and debris falling into uncovered tubes at the at the point of scale.

Table 1: Bacteriological status of different brands of ice cream

Producers	No. of samples	Total bacterial count		Coliform count		Staphylococcal count	
		CFU/ml	Log.	CFU/ml	Log.	CFU/ml	Log.
Milkvita	10	2800-3800 (3,280)	3.45-3.58 (3.52)	00	00	00	00
Igloo	10	2800-4,000 (3, 450)	3.45-3.6 (3.54)	00	00	2-7 (3.8)	0.3-0.8 (0.58)
Polar	10	10,000-19,000 (15,0000)	4.0-4.28 (4.18)	4.08-18 (11.6)	0.6-1.26 (1.06)	00	00
Savoy	10	26,000-56,000 (42,460)	4.43-4.75 (4.63)	18-42 (28)	1.26-1.62 (1.54)	7-17 (11.2)	0.8-1.23 (1.05)

The highest Coliform count was recorded with the ice cream of Savoy (28 CFU/ml) followed by Polar (11.6 CFU/ml) and lowest (nil) in the samples of Milk vita and Igloo. The results of the samples of Savoy and Polar are consistent with that reported by Tampieri (1967). His report showed that the ice cream contained > 10 Coliform per ml. The Coliform standards for ice cream should not over 10/ml (Frazier,1958 and James,1978). So, this study demonstrated that the samples of Milkvita and Igloo met the recommen -ded criteria of USPHS (1965). Hence it could be taken into consideration as superior quality ice cream. The average Staphylococcal count in ice cream sample of Igloo was found lower (3.8 CFU/ml) than the sample of Savoy (11.2 CFU/ml). On the other hand, in case of Milkvita and Polar, the Staphylococcal counts were found negative. Pelczar *et al.* (1965) stated that Staphylococci might be entrance into milk product from food handlers either suffering from acute pyogenic infections or being at a state of healthy carriers harboring the organism--s in nose or throat.

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Studies on Changes in Some of Blood Constituents of Adult Cross-bred Cattle Fed Different Levels of Extracted Rice Bran

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Abstract: Feeding of two levels of extracted rice bran (ERB) along with wheat straw was studied in 6 adult crossbred cattle. After 121 days feeding of restricted - ERB the first phase of blood sample was collected from all the animals. Subsequently the animals were shifted to *ad lib.* feeding of ERB and the second phase of blood sample was collected after 42 days. The blood samples were analysed for biochemical constituents like haemoglobin, glucose, protein, albumin, globulin, inorganic phosphorus, AST (GOT), ALT(GPT), ACP, ALP, cholesterol, creatinine and urea nitrogen. The mean values of the blood biochemical constituents in the same order were 11.10 ± 0.17 and 10.41 ± 0.16 g/100ml., 56.99 ± 0.48 and 62.70 ± 0.97 mg/100ml., 7.20 ± 0.10 and 7.80 ± 0.20g/100ml., 2.63 ± 0.07 and 2.07 ± 0.06g/100ml., 4.52 ± 0.15 and 5.65 ± 0.23g/100ml., 5.81 ± 0.08 and 7.02 ± 0.07 mg/100ml., 52.02 ± 1.93 and 59.38 ± 1.23 i.u., 20.63 ± 0.61 and 27.99 ± 1.34 i.u., 40.80 ± 4.61 and 56.53 ± 5.67 i.u., 8.03 ± 0.72 and 11.78 ± 0.77 i.u., 94.04 ± 2.36 and 79.24 ± 1.59mg/100ml., 2.00 ± 0.17 and 1.00 ± 0.03 mg/100ml., and 15.02 ± 0.67 and 33.14 ± 0.67mg/100ml. for restricted - and *ad lib.*-ERB fed animals respectively. All the mean values were differed significantly due to level of bran feeding.

Key words: Crossbred cattle, extracted rice bran, nutrient intake, enzymes, blood biochemical constituents

Introduction

Nutritional imbalances and excesses of farm animals are highly correlated with the turnover of fluids, salts and soluble organic materials in the system which reflected upon the secretions and excretions from the body and internal circulatory medium. Blood is a fluid connective tissue that is circulated throughout the vascular system carrying the vital factors to all cells in the body and serves as a principal transport medium for nutrients which also receives the waste products of nutrient metabolism from the tissue. Thus, blood biochemical screening in the nutritional research can act as an essential supportive clue for more accurate and reliable diagnosis of various physiological disorders and these pictures should be comprehensively interpreted by correlating with other nutritional parameters. Any disturbance on basic physiological process of the animal by dietary changes may lead to a chain reaction resulting in disruption of normal physiological activity and subsequently may also change the biochemical constituents of the body fluid (Sharma, 1973). In the present study, therefore, feeding of extracted rice bran at 2 levels (restricted and *ad libitum*) on the changes of blood biochemical profile was assessed.

Materials and Methods

Housing and Feeding: Healthy, 6 adult cross bred cattle of about 6½ years of age are freely given wheat straw (WS) as a basal diet with restricted amount of extracted rice bran to meet maintenance requirement of crude protein (CP) according to NRC (1989). After 121 days of experimental feeding trial the first phase of blood sample was collected from all the animals. Subsequently all the experimental animals were shifted to *ad libitum* feeding of ERB along with restricted amount of WS and the second phase of blood samples were collected after 42 days of feeding trial. In addition during the experimental feeding periods 1Kg of available green fodder was also offered as a carotene supplement to each animal throughout the experimental period. Clean drinking water was offered twice a day to all animals under uniform managerial conditions. The animals prior to experimentation were vaccinated for all common epidemic viral and bacterial diseases and twice dewormed against the helminths (albendazole @ 7.5 mg/kg B.W.).

Blood Collection: From all the experimental animals, the blood samples were collected by venipuncturing from jugular vein with a sterile, 16 guage dry needle and clean, prepackaged plastic syringe in the morning prior to feeding and watering. The blood samples were collected in centrifuge tube containing an anticoagulant (disodium EDTA and sodium fluoride; Prasad 1992). It was then centrifuged at 3000 rpm of 15 minutes to separate the plasma. The separated plasma was then transferred into previously numbered plastic vials with the help of automatic microlit pipette capped and preserved at −20°C for further analysis. About 2 ml of anticoagulanted whole blood sample was also preserved for haemoglobin estimation. Though salt-EDTA is the anticoagulant of best the choice for most haematological procedures which caused shrinkage of RBC and highly interfered with determination of non protein nitrogen, urea-N, creatinine, alkaline phosphatase (Fraser, 1993). For serum separation, about 15 ml collected whole blood in the centrifuge tube was allowed to clot for about 30 minutes then rimmed with an applicator stick and centrifuged exactly at 2000 rpm for 10 minutes. The supernatant serum was carefully aspirated and placed in the clean & labeled plastic vial and preserved in deep freeze for further assay.

Table 1: Chemical composition of experimental feeds on % DM basis

Attributes	ERB	WS
Organic matter (OM)	84.46	90.15
Crude protein (CP)	16.65	3.64
Ether extract (EE)	0.81	1.16
Total carbohydrates (TCHO)	67.00	85.35
Neutral detergent fibre (NDF)	52.54	74.66
Acid detergent fibre (ADF)	26.08	50.38
Hemi cellulose (HC)	26.48	24.28
Total ash (TA)	15.54	9.85
Acid insoluble ash (AIA)	5.74	5.81
Phosphorus (P)	1.21	0.12

ERB : Extracted rice bran,
WS : Wheat straw.

Analysis: The chemical composition of extracted rice bran and wheat straw samples was determined as per the methods detailed in AOAC (1990) and the fibre fractions viz. neutral detergent fibre (NDF), acid detergent fibre (ADF) and hemicellulose (HC) as outlined by Van Soest *et al.* (1991). The blood samples were estimated for blood biochemical constituents like AST and ALT, ALP, ACP glucose, haemoglobin, total protein, albumin, inorganic

Table 2: Mean body weight and nutrient intake of experimental animals at different levels of ERB feeding

Attributes	ERB-restricted	ERB *ad-libitum*
Body weight (BW)		
Mean BW (kg)	370.12 ± 20.41	375.88 ± 21.03
Metabolic body size ($KgW^{0.75}$)	84.31 ± 3.52	85.29 ± 3.61
Dry matter intake (DMI)		
Wheat starw, (kg/d)**	3.91 ± 0.11^{b}	1.58 ± 0.05^{a}
ERB, (kg/d)**	2.07 ± 0.09^{b}	9.94 ± 0.30^{a}
Total DMI, (kg/d)**	5.98 ± 0.17^{b}	11.52 ± 0.35^{a}
% of BW**	1.62 ± 0.05^{b}	3.08 ± 0.10^{a}
$g/KgW^{0.75}/d$**	70.99 ± 1.41^{b}	135.36 ± 2.49^{a}
Straw : ERB ratio**	65.30 : 34.70^{a} (±0.75)	13.75 : 86.25^{b} (±0.85)
Crude protein intake (CPI)		
Total CPI, (g/d)**	486.99 ± 16.98^{b}	1723.36 ± 45.02^{a}
g/100kg BW/d**	132.05 ± 2.91^{b}	461.16 ± 16.51^{a}
$g/KgW^{0.75}/d$**	5.78 ± 0.08^{b}	20.26 ± 0.45^{a}
Organic matter intake (OMI)		
Total OMI, (g/d)**	5270.27 ± 147.82^{b}	9813.78 ± 293.73^{a}
Ether extract intake (EEI)		
Total EEI, (g/d)**	63.27 ± 1.66^{b}	91.23 ± 2.86^{a}
Carbohydrate intake (CHOI)		
Total CHOI, (g/d)**	4720.15 ± 130.56^{b}	7999.12 ± 248.18^{a}
Neutral detergent fibre intake (NDFI)		
Total NDFI, (g/d)**	3999.23 ± 110.85^{b}	6716.30 ± 219.44^{a}
Acid detergent fibre intake (ADFI)		
Total ADFI, (g/d)**	2486.48 ± 69.30^{b}	3413.81 ± 126.74^{a}
Hemi cellulose intake (HCI)		
Total HCI, (g/d)**	1503.64 ± 42.20^{b}	3289.20 ± 90.45^{a}

Mean values bearing different superscripts in a row differ signifcantly. ** $P<0.01$ (highly significant effect); Non significant effect ($P<0.05$).

phosphorus, cholesterol, creatinine, urea-N as per the method described by Reitman and Frankel (1957), Kind and king (1954), Bergmeyer (1984), Cooper and Mc Daniel (1970), Wong (1928), Hiller *et al.* (1927), Gustafsson (1976), Fiske and Subba Rao (1925), Zlatkis *et al.* (1953), Wootton (1974), Rahmatullah and Boyde (1980) respectively. The data pertaining to dry matter intake, nutrient intake and blood biochemical profile was analysed for variance and tested for statistical significance employing Duncan Multiple Range Test (Snedecor and Cochran, 1994).

Results and Discussion

The chemical composition of feeds offered to the experimental animals under two different feeding systems is given in Table 1. The proximate compositions and the fibre fraction of extracted rice bran (ERB) and wheat straw (WS) were within the normal range of common values for these feeds (Ranjhan, 1991; Prakash and Ramanathan, 1995). Average metabolic body size of the animals was 84.31 ± 3.52kg and 85.29 ± 3.61kg in restricted- and *ad lib.*-ERB fed groups respectively which showed a little fluctuation but statistically non significant effect between the groups. Such fluctuation in the mean body weight was due to shifting of animals from restricted to *ad lib.* - ERB feeding. The data on voluntary dry matter intake indicated that DMI was affected significantly by the dietary treatments. The ratio of extracted rice bran in the ration was about 35% and that of wheat straw 65% in restricted ERB fed animals but the corresponding value of the ratio in *ad lib.* ERB fed animals was 86% and 14% respectively, being significantly ($P<0.01$) different between the treatments. This indicated high palatability extracted rice bran being higher intake during *ad lib.*-ERB feeding. The intake of DM was similar to that recorded by Kishan *et al.* (1991) on feeding of high level of brans in the diet of adult buffaloes. The mean body weight and, DMI and nutrient intake per unit of live weight are presented in Table 2. The average nutrient intake of crude protein was 132.05 ± 2.91 g and 461.16 ± 16.51 g per 100 kg B.W. equivalent to 5.78 ± 0.08 g and 20.26 ± 0.45 g per kg metabolic body size of restricted and *ad lib.* -ERB fed animals respectively, which revealed statistically significant ($P<0.01$) between the dietary treatments. In the present study, the nutrients intake of various proximate principles and fibre fractions like CHO, OM, EE, NDF, ADF and HC were significantly higher in *ad libitum* -ERB fed animals than that of restricted -ERB fed animals and this was in agreement to the previous reported values (Moran, 1983; Weston, 1985).

The mean values of haemoglobin level in blood observed some variation during the experimentation of dietary treatments. The difference between the ERB-restricted and *ad libitum* feeding of animals was statistically significant ($P<0.05$) but the values in both groups were within the normal range (Greatorex, 1957). The mean concentration of glucose content in the blood plasma was 56.99 ± 0.48 and 62.70 ± 0.97 mg/100ml for ERB -restricted and *ad lib.* feeding animal groups respectively, and comparable between the treatment groups which evinced significant increase in *ad lib.* feeding of ERB group, though the values were within the upper normal limit reported by Boyd (1984). This was due to higher niacin intake in ERB *ad lib.* group which results in increased level of glucose and protein (Horner *et al.*, 1989). The activity of enzyme transaminase and phosphatase gradually increased from restricted to *ad lib.* feeding of ERB group of animals. Similar finding was also reported by Prasad *et al.* (1990). The pattern of changes in the value of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and acid phosphatase (ACP) may be considered normal and little relationship with the health of the animal particularly liver and skeleton which was similar to the reported by Kaneko (1997). The average value of total protein concentration in the blood plasma revealed a little increase during the *ad lib.* -ERB feeding experiment and the increase was statistically significantly higher ($P<0.05$) in *ad lib.* -ERB fed group. The values of the total protein in blood plasma of the adult crossbred cattle during the first phase as well as second phase of experiment were found to be within the range of reported values (Jain, 1996). There was a sharp decrease ($P<0.01$) in the plasma globulin concentration (4.52 ± 0.15g/100ml) in ERB -restricted fed group with an increase in albumin/globulin ratio (0.59 ± 0.04) than that the value of ERB -*ad lib.* fed group i.e. 5.65 ± 0.23g/100ml and 0.37 ± 0.03. The mean value of plasma albumin was dropped in *ad lib.* -ERB fed animals which showed a little lower from the normal range recommended by Boyd (1984).

Table 3 : Mean values of blood biochemical constituents of experimental animals at different levels of ERB feeding

Attributes	ERB-restricted	ERB-*adlibitum*
Blood haemoglobin, (g/100ml)*	11.10 ± 0.17^a	10.41 ± 0.16^b
Plasma glucose, (mg/100ml)**	56.99 ± 0.48^b	62.70 ± 0.97^a
Serum - AST (GOT), (i.u)**	52.02 ± 1.93^b	59.38 ± 1.23^a
Serum - ALT (GPT), (i.u)**	20.63 ± 0.61^b	27.99 ± 1.34^a
Serum - ACP, (i.u)**	40.80 ± 4.61^b	56.53 ± 5.67^a
Serum - ALP, (i.u)**	8.03 ± 0.72^b	11.78 ± 0.77^a
Plasma total protein, (g/100ml)*	7.20 ± 1.10^b	7.80 ± 0.20^a
Plasma albumin, (g/100ml)**	2.63 ± 0.07^a	2.07 ± 0.06^b
Plasma globulin, (g/100ml)**	4.52 ± 0.15^b	5.65 ± 0.23^a
Albumin-globulin ratio**	0.59 ± 0.04^a	0.37 ± 0.03^b
Plasma inorganic phosphorus, (mg/100ml)**	5.81 ± 0.08^b	7.02 ± 0.07^a
Serum cholesterol, (mg/100ml)**	94.04 ± 2.36^a	79.24 ± 1.59^b
Serum creatinine, (mg/100ml)**	2.00 ± 0.17^a	$1.00 \pm 0,03^b$
Serum urea nitrogen, (mg/100ml)**	15.02 ± 0.67^b	33.14 ± 0.67^a

AST : Aspartate amino transferase or, GOT : Glutamic oxalo acetic transaminase
ALT : Alanine amino transferase or, GPT : Glutamic pyruvic transaminase
ALP : Alkaline phosphatase, ACP : Acid phosphatase.
Mean values bearing different superscripts in a row differ signifcantly. * $P<0.05$ (significant effect), ** $P<0.01$ (highly significant effect).

Hypo-albuminemia is a common phenomenon in the farm animals which may be a result of malnutrition, maldigestion or malabsorption (Johnson *et al.*, 1982). However, no such problem was observed in the present study. This aspect requires further study for finding out the reasons for successful variation. From the perusal of the values of plasma inorganic phosphorus concentration in Table 3, it may be seen that it was statistically significantly higher ($P<0.01$) in ERB -*ad lib.* fed animals than that of restricted -ERB fed animals. This was on account of higher intake of available phosphorus content in the bran (Moran, 1983). However, both the mean values were within the range of values reported by Singh *et al.* (1990). Mean concentration of cholesterol in blood serum was dropped abruptly in ERB -*ad lib.* fed animals. The difference was found significant ($P<0.01$). This was due to the higher intake of fibrous diets which are responsible for lowering the cholesterol content in serum (Husseini *et al.*, 1976). In the *ad lib.* -ERB fed animals, the level of serum creatinine sharply decreased and differed significantly ($P<0.01$) from the value of restricted-ERB fed animals. The reason for decreasing in creatinine level (2.00 v/s 1.00 mg/100ml) on *ad lib.* feeding of ERB fed adult crossbred cattle might have inhibited to the body creatinine production (Walker, 1960). Mean serum urea nitrogen concentration was, in different levels of ERB fed animals, within the lower and upper limit recorded by Boyd (1984). Higher blood urea or NH_3 concentration was considered in incidences of urinary obstruction or hepatic dysfunction (Kaneko, 1997), which was not evident from urination pattern of animals. Unfortunately its ralated attributes like volume of urine output, specific gravity, pH etc. could not be noted to arrive at a possible conclusion. Urea-N normally increase with the increasing intake of protein and non protein nitrogen (Huber *et al.*, 1976). During the second phase (*ad lib.*) of ERB feeding the level of mean blood urea-N increased dramatically which showed significantly ($P<0.01$) different. Almost doubling of blood urea-N level (15.02 v/s 32.14mg/100ml.) is an indicator of excess protein intake associated with less energy intake due to which ammonia (NH_3) released in rumen fermentation could not be efficiently utilized by the rumen microorganisms. It may be inferred from this study that feeding of different levels of extracted rice bran in adult cross-bred cattle has changed in the blood biochemical constituents.

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Knowledge, Attitude and Practices of Patients Visiting a Diabetes Care Unit

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Abstract: All patients if given proper guidance and education regarding diabetes care would be able to make significant improvement in their life-style which is helpful for good glycemic control. Education to diabetic patients would be more effective if we know the level of knowledge, attitude and practices of our patients. Thus a study was conducted to assess the general characteristics, knowledge, attitude and practices of type 2 diabetic patients attending the Out-Patient Department (OPD) of Baqai Institute of Diabetology and Endocrinology (Karachi, Pakistan). Fifty-seven percent of the patients were overweight or obese. Only 10.7% had good glycemic control. Sixty seven percent did not do exercise of any kind. The overall awareness about the risk of complications was satisfactory but the misconceptions regarding diet, insulin and diabetes were quite common. This study highlights the need for better health information to the patient through large scale awareness programmes so as to change the attitude of our public regarding diabetes.

Keywords: KAP, diabetes, education

Introduction

Prevalence of Type 2 diabetes is increasing globally (Kuller,1997) and the rate of increase is higher in the developing countries (Grol, 1997). According to a survey done by the Diabetic Association of Pakistan prevalence of diabetes in Pakistan is around 10% and an equal number of people are suffering from impaired glucose tolerance (Samad Shera, 1999). According to WHO estimates with the rising trend of diabetes seen world-wide Pakistan is currently 8th in the world ranking of diabetes and will become 4th by the year 2025 with nearly 14.3 million diabetics (Murray, 1996.). Mortality and morbidity related to diabetic complications poses great threat and burden to a nation's economy (Lorenz, 1986). Thus educating the patient is essential for prevention of complications (Lantion-Ang, 2000).

All patients if given proper guidance and education regarding diabetes care should be able to make significant improvements in their life style which would be helpful in maintaining good glycemic control. Patient's lack of understanding or attitude hinders proper guidance about disease. Even in developed countries it has been observed that improper guidance and communication could lead to poor compliance (Nutrition Sub-Committee Of the British Diabetic Association's Medical Advisory Committee, 1982). Education is likely to be effective if we know the characteristics of the patients in terms of knowledge, their attitude and practices about diabetes. For planning effective education programs identification of vulnerable groups and characteristics of the sufferers provides useful information. In a developing country like Pakistan where literacy rates are low, the chances of improper guidance about disease due to lack of understanding of patients characteristics i.e. the personality and attitude of the patients are high. Thus this study was planned to study the general characteristics and diabetes related knowledge, attitude and practices of Type 2 Diabetic patients attending a Diabetes Care Unit in Karachi.

Materials and Methods

Subjects and Sampling: One hundred Type 2 diabetic patients seen by the dietician sequentially for the first time during the period from July to September 2000.

Data Collection: Data regarding patient's characteristics, knowledge, attitude and practices was collected through questionnaire administered individually by the dietitians. The questionnaire contained queries about patient's general characteristics e.g., age, sex, family size; their knowledge of diabetes and their self-care practices such as dietary habits, exercise pattern and home monitoring of blood glucose. Anthropometric assessment included measurement of height and weight. Information regarding HbA1c, Total Blood Cholesterol (TBC) and Fasting Blood Triglyceride (FBTG) levels were obtained from medical records.

Data Entry and analysis: Data was entered and analysed on SPSS 10. Cut-off points used for categorising the patients according to various characteristics were as given below

Characteristics:

Body Mass Index: Patients having a BMI of greater than 40 were considered as very obese, those having BMI between 31-40 were considered obese, between 26- 30 were overweight, between 19-25 were normal and less than 19 were considered underweight.

Glycemic control: Patients with HbA1c between 4 to 6.4% , 6.5 - 7.5% and > 7.5% were considered as having good, fair and poor glycemic control respectively.

Cholesterol levels: Patients having total plasma cholesterol levels between 150-200 mg/dl, 201-250 mg/dl and > 250 mg/dl were regarded as having normal, high and very high cholesterol levels respectively.

Triglyceride levels: Patients having fasting plasma triglyceride levels between 50-150 mg/dl were regarded as having normal, between 151-250 mg/dl as having high and more than 250 mg/dl as having very high levels of triglycerides respectively.

Assessment of Knowledge: A questionnaire was designed to assess the diabetic knowledge of the patients and it contained 17 questions about Diet, Insulin and Diabetes. One point was given for each correct answer. A total score of 12 or above was taken as having good knowledge, 6-11 was assessed as having fair knowledge and less than 6 meant having poor knowledge about diabetes.

Diet and Exercise practice: Diet was assessed by dietician by asking about the major food groups taken by the patient. Exercise practice was assessed by inquiring about the nature of exercise done like, walking, brisk walking, jogging, swimming or weight lifting etc. The patients were also asked about the duration of their

exercises. Duration of exercise was assessed in terms of less than 15 minutes, 15-30 minutes, 30-60 minutes and more than one hour daily. Sedentary life style involved office work and not much walking around during normal work. while no exercise but active life style indicate walking and climbing stairs or extensive outdoor work during normal/routine work.

Results

Characteristics of the Subjects: The mean age of men and women was 50 and 52 respectively. Most of them had at least nine years of school education and average family income was 15,000 to 17,000 per month. (Table 1)

Weight status: Around 57% of the patients were either overweight or obese and prevalence was much higher in women as compared to men (70% Females while 52% Males). Out of those patients who were either overweight or obese only 21 patients (38%) considered themselves overweight or obese. Greater BMI with co-relation to poor glycemic control was comparative in females only.

HbA1c, Cholesterol and Triglycerides levels: Information regarding HbA1c and blood lipids was available for a smaller group of subjects as all the patients did not have their blood tests done. Around 2/3rd of the patients had their HbA1c done. Only 8 patients (11%) had good glycemic control while 57 patients (76%) had poor glycemic control. (Table 2)

Total Blood Cholesterol levels of 73 patients were assessed , out of which 40 (55%) had normal blood cholesterol levels while the rest (45%) had high or very high cholesterol levels.

Seventy one percent of the patients had their triglyceride levels tested; out of which 34 patients (48%) had normal triglyceride levels while the rest (52%) had high or very high Triglyceride levels. (Table 2)

Self Care Practices

Blood Glucose Monitoring: Sixty nine patients had glucometers but only 14 patients did Home Blood Glucose Monitoring (HBGM) regularly (daily or more than twice a week). Despite having glucometers 19 patients never did Home Blood Glucose Monitoring. As regards Lab testing only 8 patients did lab testing once in 1-2 weeks, where as 12 patients never did lab testing. (Table 3a)

Exercise practice: Thirty five patients had very sedentary life style i.e. their work as well as leisure activities both did not involve much physical activity. Only 9 patients exercised for more than half an hour daily. Females were less active than males. While 32 patients did not exercise at all but reported to have an active life style (outdoor work involving walking and climbing stairs etc.). (Table 3b)

Dietary Practices: Fruit and vegetable intake was very low as only 47% had any fruit and vegetable intake daily. Intake of sugar or sweet foods was not frequent as only 16 patients daily consumed food containing table sugar. High blood cholesterol levels was associated with low consumption of fruits. To assess the dietary indulgence the frequency of patients attending parties, lunch and dinners was seen. Majority of the patients did not attend parties frequently. Thirteen patients attended parties twice a week or more. Two patients avoided going to parties, lunch or dinner.

Knowledge: Overall knowledge regarding diabetes was not very good. Around 54% had poor knowledge about diabetes. Thirty-four percent had fair knowledge about diabetes while only 13% had good knowledge. (Table 4).

Table 1: Characteristics of the Sample

Parameters	Female	Male
Age in years	51.8 ± 11	50.1 ± 9
Number of family members	6.5 ± 2.5	6.8 ± 2.9
Years of formal Education	7.9 ± 4	10.9 ± 3
Income per month	15.0 ± 9	17.5 ± 11
Body Mass Index	28.4 ± 6	25.4 ± 4
Duration of Diabetes in years	7.5 ± 5	10.5 ± 16

Table 2: Biochemical assessment of Health Status

Parameters	Level	Percentage
HbA1c	Good	10.7
	Fair	13.3
	Poor	76.0
Cholesterol Levels	Normal	54.8
	High	37.0
	Very High	8.2
Triglyceride Levels	Normal	47.9
	High	28.2
	Very High	23.9

Table 3a : Self Care -Blood Glucose Monitoring

Type of Monitoring	Frequency	No.	Percent
Home Monitoring	Don't have glucometers	31	31
Blood Glucose	Daily or more than twice a week	14	14
	Twice or thrice a month	15	15
	Once a month or once in two months	9	9
	Very Occasionally	12	12
	Never	19	19
Laboratory Test	Once a week or once in two weeks	8	8
	Once a month or once in two months	16	16
	Thrice a year	11	11
	No regularity	53	53
	Never	12	12
	Total	100	100

Table 3b: Self care –Exercise Practice

Exercise Practice	Frequency	Percent
Sedentary life style	35	35
No exercise but active	32	32
Less than 15 minutes of exercise	13	13
15 –30 minutes of exercise	11	11
30-60 minutes of exercise	5	5
One hour of daily exercise	4	4
Total	100	100

Table 3c : Self-care diet

	Once or Twice a day	Twice or Thrice a week	Once a week	More than once a week	Totally avoids
Fruits	47%	27%	11%	15%	0%
Vegetables	41%	26%	21%	11%	0%
Meat	47%	25%	15%	12%	1%
Sugar	16%	13%	11%	6%	54%

Discussion

The management of Diabetes Mellitus not only requires the prescription of the appropriate nutritional and pharmacological regimen by the physician but also intensive education and counselling of the patient (Nuttall, 1993).

Control of obesity and ideal body weight is important for better glycemic control and prevention of complications, but the characteristics of our patients were not according to this norm as more than half of the patients were overweight or obese. Majority of the patients had a wrong assessment of their own weights and most overweight patients did not consider themselves to be overweight, thus a problem with their attitudes. Thus the results of this study highlights the need to educate the patients about their body weight as well as assessment of obesity.

Greater BMI with co-relation to poor glycemic control was comparative in females only. It was not easy for everybody to understand the concept of Body Mass Index and it was suggested that waist circumference may be used as a crude parameter

Table 4: Knowledge about diabetes

Misconceptions	Agree%
Insulin in the last treatment and it should be avoided as much as possible	42
Insulin is an addiction	25
The dose of insulin keeps on increasing and at one stage it stops to work	35
Insulin causes severe hypos	35
If strict diet control is done, insulin can be avoided	37
Human insulin can be extracted from human pancreas	15
If someone experiences hypos or an infection insulin can be stopped	28
Karela Water and Jaman seeds are very effective in reducing blood sugar	64
Under root vegetables are not allowed in diabetes	65
Besan and Channa reduces blood sugar	64
Ghee increases weight but oil does not	34
Milk should not be taken during infections	22
Diabetes is caused by a Diabetic virus	7
Diabetes is an epidemic disease	14
A bad shock can cause diabetes	43
No diet control is needed after tablets	22
If one spouse has Diabetes the other can get it	28

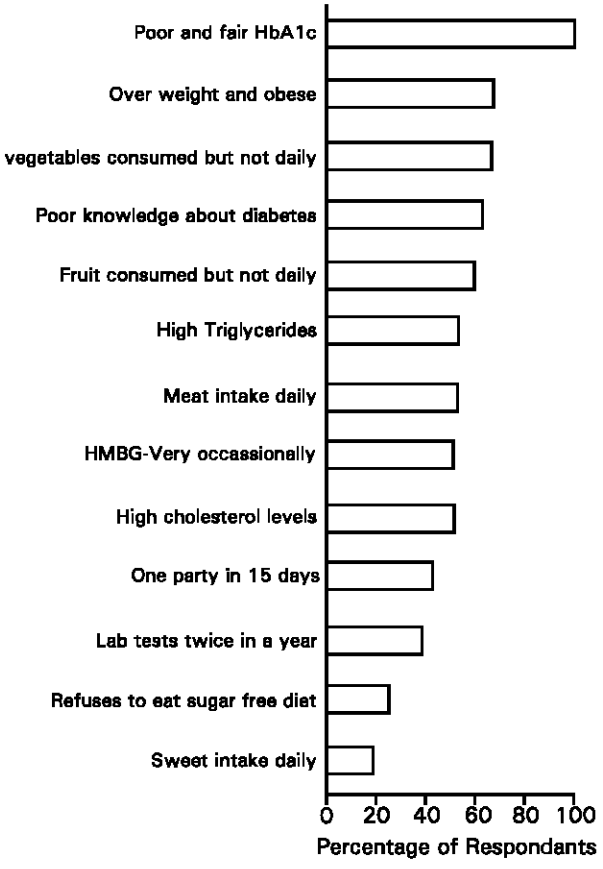

Fig. 1: Proportion of diabetics having risk indicating characteristics

instead as it is easier and more understandable. Glycemic control could be improved by a weight loss of only 10% of the initial weight and thus public education and awareness about the beneficial effects of consuming a healthy diet was required (Grol, 1997).

Concepts of healthy food consumption among patients were not clear. In general the diabetics were overweight even though they were not taking sugary foods regularly. Thus positive energy balance could probably be due to sedentary life style and high-energy intake. The source of calories appears to be starchy or fatty foods rather than simple sugars. High blood cholesterol levels was associated with low consumption of fruits. While daily vegetable consumption was not affecting blood cholesterol and glucose levels. Thus the need for education about caloric requirements and energy value of foods is required. The lack of proper knowledge as regards diet requirements of each patient should be given individual dietary advice with clear view of its purpose, so that they understand and follow it in practice.

The role of high fibre diet in improving glycemic control is well established and high fibre diet decreases the risk of diabetes (Schneider, 1997). The consumption of fruits and vegetables in our study as shown by Table 3c seems to be low which shows the poor intake of fibre and this is due to wrong dietary habits that are prevailing in our society. Improving the dietary pattern of the diabetics in our society will not be an easy task. Great efforts would be needed by health teams to enhance education of the diabetic patient in order to promote compliance with recommendations regarding diet and exercise (Aravjo, 1999). This also highlights the need of having dieticians and educators alongside consultant diabetologists in our diabetes care centres to educate the patients about diet and exercise.

Overall Exercise was also found to be poor. Only a few had good exercise practices (> 30 minutes per day). Females were less active than males. Many studies have confirmed the beneficial role of physical activity in improving glycemic control. Given the importance of physical activity to diabetes management, the low physical activity in this and similar studies should raise concerns among clinicians and it is necessary that all patients should be encouraged to increase their physical activity (Tans, 1997).

Self-monitoring of blood glucose is a simple and practical procedure acceptable for those patients who can afford it and facilitates the attainment of good glycemic control but unfortunately in our local population the practice of using glucometers was not good as although 50% of the patients had their own glucometers but only 14 patients were regularly monitoring their blood sugars. This could be due to the financial constraint of the patients as they have to purchase the costly strips. This problem can somewhat be resolved by using urine strips for glucose checking.

Education and counselling about all the aspects of diabetes is needed. Group education as well as individualised education programmes should be planned which can lead to better preventive and management techniques in diabetes.

The attitude and practices of patients studied in this study is summarised in Fig. 1 and this shows the cumulative effect of

various risk factors which can lead to poor control and complications of diabetes.

The knowledge of the subjects visiting the centre for the first time was found to be inadequate. This probably is due to inadequate information, non-availability of educational material and improper guidance. The reasons of the poor knowledge need to be further studied in detail in our population. Thus there is need for arranging large scale awareness programs for the general public and also to identify and use media to spread the message which could change the attitude of our public in the future.

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