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The energy constituents and lipidaemic effects of various formulations of cocoyam-Bambara groundnut-soya bean flour blends fed on streptozotocin-induced diabetic rats

Henry Chukwuemeka Uro-Chukwu [1, 2, 3, *], Franklyn Chidiebere Uro-Chukwu [3] and Frances Chidinma Uro-Chukwu [3]

[1] *Department of Medical Biochemistry Alex Ekwueme Federal University, Ndufu-Alike, Ikwo, Nigeria.*
[2] *Department of Community Medicine, Ebonyi State University, Abakaliki, Nigeria*
[3] *Institute For Nutrition, Nutraceuticals & Public Health Research & Development, Nigeria*

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Abstract

Background: Diabetes mellitus is a chronic disease affecting a reasonable percentage of people globally, recording high incidence, chronicity and mortality. There are challenges in curbing the burden due to high care cost resulting in adopting cost-saving strategies through use of plant foods. High calorie diets and legumes including culturally acceptable staple foods like cocoyam, soya bean and Bambara groundnut are undergoing experimental studies for their clinical application in the management of T2DM. This study is focusing on understanding the relationship between high calorie diets and hyperlipidaemia, due to the relevance of such knowledge in cardiovascular diseases.

Methodology: Three commonly consumed plant foods: Cocoyam (CY), Bambara groundnut (BGN) and Soya bean (SB) were obtained, processed, and pelletized after samples of the flour were subjected to proximate analysis and energy content calculation. The intervention feeds were given for 28 days to the seven groups of male albino rats that were induced with diabetes using Streptozotocin, Blood specimen was collected, from the intervention groups and controls for lipid profile determination.

Results & Discussion: SPSS and one-way ANOVA were used to analyze the generated data, while the Turkey`s post-hoc test, was applied to detect the difference in mean, with the significant difference pegged at $p<0.05$ value. Group A treated with Formulation 1 (16.6%CY: 16.6%SB: 16.6%BGN: 50%RF), which in comparison to the diabetic control, reduced the Total cholesterol (TC) level by 12.53% and the Low-density lipoprotein cholesterol (LDLc) levels by 24.71%. Formulations 1`s 16.3kJ/g and commercial rat feed (RF)`s 16.7kJ/g energy values were comparable and with 65.7% higher fibre content compared to RF, might have contributed to its hypocholesterolaemic potency. Formulation 7, a binary blend (50%SB + 50% RF), with the highest fat content (19.55%) and highest energy value (23.8kJ/g), had comparatively lower potency than formulation 1 and RF.

Conclusion: CY-BGN-SB flour blend formulations contained caloric values that approximated that of commercial rat foods and despite the higher fat content in some of the formulations they all exerted hypolipidaemic effects possibly due to reasonable number of dietary fibres and hypoglycemic-bioactive constituents, and hence can be recommended for nutritional management of diabetes especially if human studies are conducted.

Keywords: Caloric Values; Lipogenic Substances; Medicinal Plant Foods; Diabetic Rats

* Corresponding author: Uro-Chukwu, Henry Chukwuemeka.

1. Introduction

Diabetes mellitus is a chronic disease affecting a reasonable percentage of people globally, recording high incidence, chronicity and mortality [1]. Due to the multisystem effects, diabetes presents with varying symptoms that finally results in organ damage [2]. In developing countries much more number of the productive populations are affected when compared to the advanced countries [3]. It is reported that multifarious factors ranging from lack of health personnel, outdated medical equipment and infrastructure and fragile health system are contributory to the high prevalence of poorly treated cases in poor countries [4, 5, 6]. Efforts to counter such unacceptable disease burden have made incursions into changes in lifestyle, dietary intake and improved physical activities [7]. Management of T2DM has therefore included nutritional component for effective results [8, 9], with such strategies encouraged by the World Health Organization [10]. Experts have reported the presence of bioactive compounds in medicinal plant foods, with research showing the biological activities of such constituents [11, 12]. Immunomodulatory, anti-inflammation, antihypercholesterolaemic and antihyperglycemia are some of the documented bioactivities of these biochemical constituents [11, 12]. Among the bioactive compounds in food plants are polyphenols, flavonoids, peptides and amino acids whose actions influence several biochemical pathways [13]. Some of these plant foods with biochemical activities and reported mechanisms of action are abundant in Africa and Asian continents and include cocoyam [14-16], Bambara groundnut (*vigna subterranean*) [17-31] and soya bean (*Glycine max. (L) Merrill*) [32-36]. Various studies have been carried out on to validate the documented mechanisms through these bioactive compounds exert their biochemical effects *invivo* and *invitro* [37-46]. One of the considered criteria for use of dietary strategies in T2DM management is the derivable energy from the food source. In the administration of single item plant foods such as unripe plantain [47 – 50], cocoyam [51 – 54], soya bean [54 -56], bambara groundnut [57-60], the energy content of the plant foods was not determined since these food plants were regarded as high calorie diets. The effects on the lipid profile were reported in some of the studies [55, 56, 58,60, 61] Since these plant foods are usually consumed in combinations, and the various studies on flour blends didn't report the energy content [61-70], with only few stating the lipidaemic effects [61,62,65, 66], this study intended to evaluate the energy constituents of the CYN-BGN-SB flour blends and the lipidaemic effects in SIDRs treated with such flour blends, so as to take into cognizance possible metabolic markers and cardiovascular risk in administering such blends in diabetic and hypertensive patients.

1.1. Collection and Preparation of the Plant Food Material

The seeds of Bambara groundnut and soya bean together with cocoyam roots were purchased, washed, peeled (in the case of cocoyam) and soaked in water for 10 minutes, boiled, oven-dried at 60^0C before processing into fine flour and pellets, which were stored in air-tight containers.

1.2. Proximate Analysis of the flour formulations (AOAC, 1990)

The moisture, crude protein, lipid, crude fibre and ash contents of the various flour formulation were conducted using the AOAC method [75]. Samples were made in triplicates, incinerated in a muffle furnace at 600^0C. When the weight obtained was constant, the total carbohydrate (CHO) content was obtained by subtracting the sum of the % moisture, % ash, % lipid and % crude protein from 100%, while the energy value of the test feeds was calculated from the Atwater formula by multiplying (the total CHO, + % crude protein) by 4, and (% lipid) x 9 respectively and adding up the values of the three.

1.3. Experimental Animals

Male albino rats numbering 85 and with weight range: 134 – 249 grams were purchased and kept according to their weight similarity, with each set containing eight rats in one cage and the rats differentiated with circular ink marks numbered 1-8, on the tail. The total number of sets were ten designated as Groups A-J. Of these groups were ones designated as standard, normal and negative control groups the intervention groups, with each group placed on formulations as captured in Table 1. The intervention formulation were based on similar reported experiments [62-64, 68-70]. All the rats were acclimatized for one week within which time the weights were recorded while commercial rats' feeds and water were given *ad libitum*. The standard procedures for handling experimental animals were strictly followed [71].

1.4. Induction of Insulin Resistance Using Low Fructose Diet

On the last day of the acclimatization, insulin resistance induction was commenced by administering 10 grams of fructose dissolved in 300 mL of water *ad libitum*, to the rats for one week while feeding the rats on the commercial feeds.

1.5. Induction of Type 2 Diabetes Mellitus using STZ

At the end of the 6^{th} day of placing the rats on low-dose fructose diet, the rats were fasted overnight before collecting blood samples for fasting blood glucose estimation. From the FBG results, the normoglycemic rats were injected with Streptozotocin (STZ). The STZ was prepared by dissolving 1 gram of the drug in 50 mL of freshly prepared sodium citrate buffer, 0.1M, pH 4.5), at a dose of 45 mg/kg [72, 73]. At day 6 and day 12 post-STZ peritoneal injection, blood samples were collected from the tails of the rat for estimation of blood glucose concentrations. Results from the blood glucose concentration was used to identify the rats that had elevated levels and assigned randomly into the groups A, B, D-J, while Group C was specifically assigned to the normoglycemic rats not treated with STZ. All the rat groups were subjected to intervention formulations as detailed in Table 1, using formulations reported in previous work [74].

Table 1 The Formulations and Their Compositions given to Each Rat Group

Rat Groups	Formulations	Formulation Compositions
A	1	16.6%CY: 16.6%SB: 16.6%BGN: 50%RF
B	Standard Control	Metformin + 100% Rat Feed (RF)
C	Normal Control	100% RF
D	Negative Control	100% RF
E	2	12.5%CY: 12.5%SB: 25%BGN: 50%RF
F	4	12.5%CY: 25%SB: 12.5%BGN: 50%RF
G	7	0%CY: 50%SB: 0%BGN: 50%RF
H	5	0%CY: 0%SB: 50%BGN: 50%RF
I	6	50%CY: 0%SB: 0%BGN: 50%RF
J	3	25%CY: 12.5%SB: 12.5%BGN: 50%RF

RF =Rat Feed
CY = Cocoyam
SB = Soya Bean
BGN = Bambara Groundnut

1.6. Estimation of Serum Lipids

The anti-diabetic drug, metformin and the CY-BGN-SB blend formulations were given to the various rat groups for 28 days, at the end of which blood samples from the veins in the tails of the rats were collected to assess for total cholesterol and High-Density Lipoprotein Cholesterol concentration, with the aid of Randox assay diagnostic kits [76]. To determine the LDLc and VLDLc concentration, Frieddewald and colleagues' formula was applied, in which LDLc = Total cholesterol – (HDLc + [TG/5]) [77].

1.7. Statistical Analysis

The Statistical Package for Social Sciences (SPSS) (IBM SPSS, version 20 Chicago, USA). Was used to enter the collected data, using the double-check entry approach to ensure data entry accuracy. The mean recorded from the different groups, were compared using the one-way Analysis of variance (ANOVA) was adopted for comparison of the mean of the various groups, taking into consideration the group independence, the response variable and its normality with the homogeneity of the variance. The Tukey`s post-hoc test was used to ascertain the significant difference of the pairs, based on the detailed pairwise comparison of all group combinations. A 5% level of significance was employed in all the tests. Based on the results, bar charts of the serum lipid profile were constructed.

2. Results

2.1. Result of the Proximate Analysis of Pilot Feed Formulations

Proximate analysis of food extracts helps to calculate the energy content of intervention formulations to ensure similarity of the quantity of energy administered to the rat groups. The proximate analysis of these formulations are contained in Table 2 below.

Table 2 Proximate Composition and calculated Energy Content of the rat feed formulations

Formulations	Codes	Moisture (%) (Mean ±SEM)	Ash (%) (Mean ±SEM)	Fiber (%) (Mean ±SEM)	Protein (%) (Mean ±SEM)	Fat (%) (Mean ±SEM)	*CHO (%) (Mean ±SEM)	Energy Content (kCal)/100g	Energy Content (kcal)/ gram	Energy Content (kJ)/ gram
1	4	5.92 (0.08)	5.78 (0.75)	2.90 (0.06)	18.57 (1.68)	9.93 (0.32)	56.91 (1.23)	391.29 (0.38)	3.91 (0.25)	16.37 (0.01)
2	6	7.82 (0.71)	6.04 (0.77)	3.04 (0.25)	18.57 (1.09)	11.67 (0.84)	53.17 (1.03)	391.99 (0.47)	3.92 (0.18)	16.40 (0.08)
3	3B	7.12 (0.10)	4.22 (0.45)	3.59 (0.39)	16.73 (0.90)	7.90 (0.51)	60.05 (0.90)	378.22 (0.54)	3.78 (0.18)	15.82 (0.41)
4	5	7.18 (1.25)	3.99 (0.39)	3.73 (0.31)	19.72 (1.03)	11.66 (0.77)	53.73 (0.83)	398.74 (0.06)	3.99 (0.00)	16.68 (0.05)
5	0	6.92 (0.13)	3.34 (0.18)	1.54 (0.28)	9.24 (0.51)	4.98 (0.14)	73.99 (1.25)	377.74 (0.34)	3.78 (0.51)	15.80 (0.33)
6	CY	5.52 (0.13)	4.64 (0.06)	2,01 (0.25)	8.49 (0.54)	1.58 (0.06)	77.78 (1.24)	359.3 (0.39)	3.59 (0.38)	15.03 (0.09)
7	SB	2.73 (0.12)	5.32 (0.08)	1.55 (0.06)	43.42 (1.08)	19.55 (0.55)	55.05 (0.90)	569.83 (0.41)	5.70 (0.25)	23.84 (0.10)
RF	RF	6.10 (0.79)	4.65 (0.10)	1.75 (0.05)	18.34 (0.76)	9.40 (0.22)	59.78 (0.77)	397.08 (0.16)	3.97 (0.10)	16.61 (0.51)

*CHO = Carbohydrate; SB = Soya Bean; CY = Cocoyam; RF = Commercial Rat feed

Table 2 shows the proximate composition of the various flour blends and that of the commercial rat feeds (RF). Data from the proximate composition of flour blends was used to calculate the energy content of the formulations, using Artwater formula as follows: Energy (in Kcal) = 4x (Protein + CHO mass in grams) + 9 x mass of fat in grams.

2.2. Results of Serum Lipid Profile

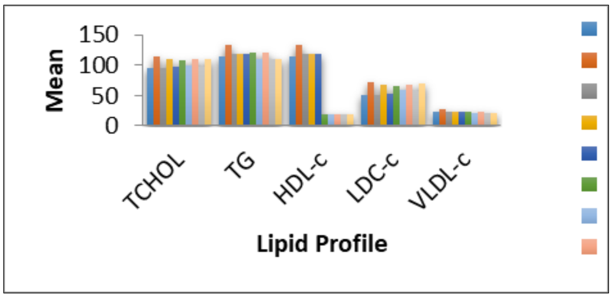

Figure 1 Bar Charts for the Serum Lipid Profile for the various diabetic and non-diabetic rat groups showing the comparable values of Groups A and C

Figure 1 is the result of mean analysis of serum lipid profile among various groups. Significant ($p<0.05$) difference among the groups were observed for the TCHOL, HDL-c and LDL-c only.

3. Discussion

3.1. The Energy Constituents of the Various Flour Blends & Its Effect on Serum Lipid Profile

The association of unhealthy foods intake with high incidence of non-communicable diseases like diabetes mellitus [78], has led to the adoption of recommended dietary pattern in diabetic management [79], with its use predating the administration of antidiabetic drugs [79, 80], even though adherence to nutritional guideline has been poor in developing countries [81]. Carbohydrates and other energy giving foods are recommended in the meal plan for diabetics but such foods` glycemic index are considered so as to achieve better clinical outcome [82]. Fats with high saturation are restricted [83] because of the higher sensitivity of T2DM patients to dietary cholesterol than the general population [84], hence only fats higher in monounsaturated fatty acid (MUFA) and polyunsaturated fatty acids (PUFA are recommended [85]. Fatty acids alter cell membrane functions, enzyme activity, insulin signaling and gene expression [86]. Proteins and its source have relationship with glucose metabolism [87] as protein-rich increase insulin secretion thereby reducing postprandial blood glucose concentration [61] depending on the source. Unlike animal source, consumption of plant-sourced proteins like soy is linked with low risk of T2DM [87, 88].

Cocoyam with its constituent protein (0.13 – 5.20g/100gDM), carbohydrate (27.64-41.68g/100gDM), and fat (0.39-4.07g/100gDM), is considered low-fat high calorie diet [89], beside phytochemicals and micronutrients [14, 15]. Bambara groundnut with its carbohydrates (57-67%), protein (15-17%) and lipids (10%) [17 – 20], in addition to micronutrients [90] and bioactive compounds [21] and anti-nutrients [91], is considered moderate fat high calorie diet, while legumes like soya bean content of 30-50g/100g proteins, 20-35g/100g carbohydrates and 15-25g/100g lipids [32, 92], with the lipid content being mainly PUFA (63%); MUFA (21.5%) and SFA (15%) [32], as well as micronutrients [33] and bioactive compounds [93] can be classified as high protein, high fat, calorie diet.

In this study, the commercial rat feed (Formulations RF) had an energy content of 3.97Kcal (16.61kJ), which approximated with Formulations 1- 6 (15kJ/gram – 17 kJ/ gram), out of which formulations 1-4, contained all the intervention flour composites, while Formulations 5 and 6 contained only single plant foods, namely: Bambara groundnut and cocoyam respectively. High soya bean intervention flour (Formulations 7), used in Group G rats, had energy content of 24kJ/gram (Table 1), implying that except the latter, all the STZ-induced diabetic rats consumed formulations with approximately equal amount of energy content (Table 2), despite the variations in their protein and fat contents, which are anticipated to have effect of the lipid profile levels of the experimental animals. From the proximate composition Table 2, the formulations 4,7,5, 6 and 3 respectively and contained 11.66% and 53.73%; 19.55% and 55.05%; 4.98% and 73.99%; 1.58% and 77.78% and 7.90% and 60.05% fat and carbohydrate content respectively, representing the highest individual contributions of lipogenesis substrates among the intervention formulations. It is possible also that such substrates might contribute to higher fat deposition in the liver

However the serum lipid analysis revealed in this study that Groups A, E and I had the least mean values of the Total Cholesterol (TC), and which approximated values obtained from the normal control. In the case of the mean values of the triglyceride and VLDLc, there was no significant difference among the groups, but the HDLc mean values varied significantly when Groups A, B, C and D were compared with Groups F, G, H, I and J, which were lower. The mean values of the LDLc, were minimal in Groups A, C and E and hence significantly lower than values obtained from the diabetic control and other groups. The study therefore revealed a raised plasma values of the TC and LDLc in the diabetic control when compared to the normal control as previously reported by scholars [94], but the significantly minimal mean values of TC and LDL in Group A which was administered with formulation 1, containing all the composite flours in moderate concentrations (16.6%CY: 16.6%SB: 16.6%BGN: 50%RF), when compared to the diabetic control, was in line with the study conducted by Wang and colleagues [94].

Dyslipidemia occurs in T2DM because such patients lipid profile levels reveals raised plasma levels of triglycerides with an accompanying reduced levels of high-density lipoproteins (HDL) and high levels of low-density lipoproteins (LDL) [95]. As the triglycerides blood levels rises, the HDLc levels are catabolized and the LDLc fraction rises [96]. Once this happens, circulating fatty acid levels are raised resulting in insulin resistance and pancreatic β-cell damage [97], furthering inhibiting the actions of lipase and worsening the elevated levels of free fatty acids [98], with its resultant negative impact on the adipose tissue and skeletal muscles [99], inhibition of metabolic enzymes, stimulation of free radical generating-enzymes and evolution of deleterious micro environment [100].

Administration of plant extract with antioxidant bioactivity, reverses these lipidaemic effects and hence improved lipid profile [94], due to presence of phytochemicals [49]. When Hydro-methanol extract of *Cocos nucifera Linn (arecaceae)* was given to SIDRs, the lipid profile improved [101]. Medicinal plant food blends involving use of unripe plantain and millet fed on diabetes rats, had positive biochemical effects [74], while the administration of Dioscorea dumentorum (bitter yam) and digitaria exiles (acha) in diabetic rats, exerted hypolipidemic effects [102]. When cocoyam-cowpea-plantain flour blend was consumed by healthy individuals the blood lipid profile improved [103], while an African yam bean (*Sphenostylis sternocarpa*)-sorghum (*Sorghum bicolor L.*)-unripe plantain (*Musa paradisiaca L.*) flour mix also produced hypolipidemic in diabetic rats [104].

The hypoglycemic effects of soya bean flour blends in diabetic animals have been variously reported in consumed dough produced from maize-soybean-millet [70]. Optimized dough meal from plantain- soybean cake-rice bran flour blend fed on STZ Induced diabetic rats [67]; matured plantain-soya cake-wheat bran flour mix [68]; from Plantain-defatted soybean-tiger nut flour mix fed on diabetic rats [69] and Cocoyam-Bamabara groundnut-soya bean given singly and in combinations [57]. In these studies, the effects of these blends on the lipid profile were not estimated. Soya bean-only formulation studies have reported hypolipidemic effects in diabetic rats [55, 56].

Previous reports on Bambara groundnut-only formulations documented its hypolipidemic effects [5758,60] similar to its flour blend. In a blend of Bambara groundnut and sweet orange peels, Oguntuase and colleges reported improved lipid profile [65]. Similarly, Ibrahim and co-researchers documented the hypolipidemic potentials of finger millet-Bambara groundnut- *Lecaniodiscus Cupanioides (*Khain) flour blends [66]. There are other combinations such as Bambara groundnut flour mixed food extracts like sorghum and butternut, with documented improved nutritional content and bioactive fibres [105].

Rather than raise the lipid profile of experimental animals and humans fed on soya bean singly or as food mix, it actually exerts a hypolipidemic effect [35] despite the high fat and protein contents. First is the presence of bioactive compounds including the high amount of arginine and low amount of methionine [106], which causes elevated nitric oxide [107] and reduced level of homocysteine [108]. Secondly, the non-digestible peptides such as Leu-Pro-Tyr-Pro-Arg, peptide derived from glycinin present in Soya bean raises the faecal excretion of steroids which enhances bile acid production, thereby increasing the number of LDLc receptors, enhanced uptake and oxidation of cholesterol in the hepatocytes [109]. These anorectic peptides also affects the appetite causing reduced food intake and fat accumulation [110] and hepatic lipotoxicity [46].Thirdly, soya bean contains isoflavones, which act as a β-oestrogen receptors in the hepatocytes enhancing cholesterol catabolism and β-oxidation of fatty acids [111]. Fourthly, the β-conglycinin and glycinin proteins in soya bean, causes higher faecal excretion of fatty acid, induction of β-oxidation and reduction of fatty acid synthesis in the hepatocytes [37]. Soya bean also contains phytate reported to negate fatty acid deposition in the liver [38], besides its anti-diabetic effects [39]. These might be reasons why despite that Formulation 7 used in Group G, with its highest calorie content when compared to other formulations, still exerted similar lipidaemic effect as other formulations

4. Conclusion

Cocoyam-bambara groundnut-soya bean flour blend, exerted hypolipidemic effects in STZ-induced diabetic rats, with the most potent effect shown in Group A treated with Formulation 1 (16.6%CY: 16.6%SB: 16.6%BGN: 50%RF), which in comparison to the diabetic control, reduced the Total cholesterol (TC) level by 12.53% and the Low-density lipoprotein cholesterol (LDLc) levels by 24.71%. Formulations 1`s 16.3kJ/g and RF`s 16.7kJ/g energy values were comparable and with 65.7% higher fibre content compared to RF, might have contributed to its hypocholesterolaemic potency. Formulation 7, a binary blend of 50% soya bean with 50% RF, with the highest fat content (19.55%) and highest energy value (23.8kJ/g), had comparatively lower potency than formulation 1 and RF. The clinical implication of these findings is that in using functional foods as treatment adjunct in T2DM, considerations should be given to food mix that could provide the needed energy, fibre content and bioactive compounds to achieve the desired biochemical effects. Human studies are therefore suggested to further increase the understanding in the use of this food blends in patients with such non-communicable diseases.

Study Limitations

Determinants of the rate of food and drink intake were beyond the researchers` control and might have acted as confounders. There were equally constitutional and genetic variables on the rat populations as well as the fluid intake dependent factors. The biochemical actions of each of the identified bioactive compounds needed to be ascertained in other to link each biochemical change to the biomolecules involved. These identified gaps demand further research.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval was obtained from The Research & Ethics Committee, Faculty of Medical Sciences, Rivers State University, Port Harcourt, Nigeria.

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